Exponent®

Pilot Study Report: Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils

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Prepared for

The Dow Chemical Company
Michigan Operations
47 Building
Midland, Michigan 48667

Prepared by

Exponent
1800 Diagonal Road
Suite 300
Alexandria, Virginia 22314

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Acronyms and Abbreviations

CV	coefficient of variability
EROD	ethoxyresorufin O-deethylase
HR-GC/MS	high-resolution gas chromatography/mass spectrometry
MROD	methoxyresorufin O-deethylase
MSU	Michigan State University
NTP	National Toxicology Program
PCDD/F	polychlorinated dibenzo-*p*-dioxin/furan
4-PeCDF	2,3,4,7,8-pentachlorodibenzofuran
RBA	relative bioavailability
RPD	relative percent difference
SOP	standard operating procedure
TCDD	tetrachlorodibenzo-*p*-dioxin

Executive Summary

The overall objective of this pilot study is to evaluate two animal models (Sprague-Dawley rats and juvenile swine) for measuring the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and the other dioxin/furan congeners of importance in soils from Midland, Michigan, and the Tittabawassee River flood plain. The study design includes a test soil from each of these two areas, because the toxic equivalent (TEQ) for dioxins/furans in Midland soils is dominated by TCDD, while that of the Tittabawassee River flood plain soils is dominated by furans (4-PeCDF in particular). The results from this pilot study will be used to complete the design of a full-scale study of dioxin/furan bioavailability from soil.

Specific objectives of the pilot study include:

- Evaluate the feasibility of detecting dioxins/furans in the tissues of rats and swine dosed with soil from Midland and the Tittabawassee River flood plain
- Evaluate the proposed study design in rats and swine for measuring the relative bioavailability of dioxins/furans in soil
- Evaluate whether five animals per dose group will be an adequate number for the full study (note that for the rats in the pilot study, 10 animals will be used, and the tissues from each pair of rats will be combined to provide 5 samples for analysis).

Each of the two soils was administered to rats in a soil/feed mixture for 30 days. Reference materials (feed and corn oil gavage) were spiked with the five most predominant TEQ-contributing congeners for each soil at concentrations designed to result in administered doses equivalent to those received in the soil/feed mixtures. Soils were administered to swine for 30 days wrapped in dough balls. The reference corn oil materials with matched doses of the five most predominant TEQ contributors for each soil were administered to swine in gelatin capsules wrapped in dough balls. At the conclusion of dosing, liver and adipose tissues were collected from experimental animals, and concentrations of the congeners of interest and EROD/MROD[1] activity in hepatic tissues were measured in all rats and swine. EROD and MROD activity was measured to evaluate whether or not differential enzyme induction (CYP1A1 and CYP1A2) was occurring between the soil and reference groups. Different levels of enzyme induction could result in different rates of metabolism or different distribution patterns between the two groups.

Relative bioavailability was estimated by comparing the fractions of administered dose retained in liver, adipose, and a combination of the two tissues between the soil and reference materials. This method relies on two assumptions. First, this method assumes that the majority of each compound would be distributed to liver and adipose tissues, and that the proportion of material distributed to other tissues would not be different between the soil and reference groups.

[1] Ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-deethylase (MROD) assays.

Second, the method assumes that the rate of elimination for each congener is the same in the soil and the reference-material group animals.

The concentrations of test compounds in both liver and adipose tissue were consistently above the detection limits in rats for both soils. In swine, tissue concentrations of congeners of interest were not consistently above detection or lower calibration limits for the Midland soil, but were consistently detectable and quantifiable in the group administered the Tittabawassee River flood plain soil, which had higher levels of contaminants.

Hepatic EROD activity was statistically significantly increased in rats in all reference-material groups compared to the respective soil groups. In swine, no statistically significant difference in EROD/MROD activity was observed between soil and reference groups for either soil.

The two animal models produced statistically significantly different estimates of relative bioavailability (RBA) for all of the congeners in the Tittabawasse River flood plain soil and for two of the congeners in the Midland soil (Figures 10 and 11). These differences may be due in substantial part to the differential induction in the rat soil and reference-material groups. Increased enzyme induction in the reference groups could result in increased metabolism rates in these groups compared to the soil groups, violating the assumption of equal elimination rates between the soil and reference groups. Increased EROD activity in the reference groups, as a marker for the CYP1A1 enzyme, would result in increased metabolism of TCDF in the reference groups compared to the soil groups, with accompanying lower retained fractions of administered dose. This would result in a false elevation of the estimated RBA in the soil groups compared to the reference groups.

Issues associated with differential enzyme induction in rats for both soils, and achieving detectable tissue concentrations in swine for the Midland soil, render most of the RBA estimates resulting from this pilot study unreliable. The swine-based RBA estimates for the Tittabawassee River flood plain soil do not suffer from either of these limitations and may provide a reliable estimate of the RBA values for this soil.

Several design modifications are recommended for future studies, in order to reduce costs, achieve detectable compound concentrations, and reduce the likelihood of differential enzyme induction between soil and reference groups. In summary, the following changes are recommended:

1. Omit the feed reference group, as results in this study confirm the general conclusion that feed has a relative bioavailability compared to corn oil gavage of about 70%. Further demonstration of this is unnecessary.

2. For purposes of reducing costs, it would be desirable to use a single animal model. Based on the results of this pilot study, either animal could be used in experiments going forward, with modifications to the study design. Pros and cons of each model are discussed in more detail in the report below, but specific considerations apply to either model:

   – If rats are used, reference material dose levels will need to be matched more closely to anticipated *absorbed* doses in the soil groups in order

to avoid differential induction of enzyme activity between soil and reference groups.

- If swine are used, the administered doses of soils with lower TEQ concentrations (for instance, Midland-area soils with TEQ concentrations at or below the levels in the soil tested in this study) will need to be increased in order to achieve reliably detectable and quantifiable tissue concentrations.

3. For purposes of reducing costs, it would be desirable to analyze only a single tissue (liver or adipose) from each test animal. Data on compound distribution from this study support use of a single tissue for either animal model, with the most consistent measures resulting from liver tissue in the rat and adipose tissue in the swine.

4. Retain hepatic EROD/MROD measurements as part of the study design, as a means of ensuring that differential induction of hepatic enzymes is not occurring in subsequent tests.

Introduction

The overall objective of this pilot study is to evaluate two animal models (Sprague-Dawley rats and juvenile swine) for measuring the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and the other dioxin/furan congeners of importance in soils from Midland, Michigan, and the Tittabawassee River flood plain. The study design includes a test soil from each of these two areas, because the toxic equivalent (TEQ) for dioxins/furans in Midland soils is dominated by TCDD, while that of the Tittabawassee River flood plain soils is dominated by furans (4-PeCDF in particular). Because the TCDD and 4-PeCDF may behave differently in these two animal models, a soil from each of these two areas was chosen for evaluation in the pilot study. The results from this pilot study will be used to complete the design of a full-scale study of dioxin/furan bioavailability from soil.

Specific objectives of the pilot study include:

- Evaluate the feasibility of detecting dioxins/furans in the tissues of rats and swine dosed with soil from Midland and the Tittabawassee River flood plain
- Evaluate the proposed study design in rats and swine for measuring the relative bioavailability of dioxins/furans in soil
- Evaluate whether five animals per dose group will be an adequate number for the full study (note that for the rats in the pilot study, 10 animals will be used, and the tissues from each pair of rats will be combined to provide 5 samples for analysis).

The study in the rat model will be used to assess the oral bioavailability of dioxins/furans from soil relative to that from both rat feed and oral gavage doses. This is warranted because relevant toxicology studies underlying estimates of cancer slope and serving as possible sources for reference doses have used both corn oil gavage and feed for administration of compounds. Thus, if dioxins/furans in soil are less bioavailable than those in rat feed, an adjustment in the risk assessment is warranted to account for this difference. In addition, the rat studies will allow for comparison to the recent National Toxicology Program (NTP) chronic carcinogenesis bioassays, in which the rats were dosed by corn oil gavage.

The swine study will be conducted to evaluate the oral bioavailability of dioxins/furans from two Midland soils in an *in vivo* model that is more similar to humans than the rat. The results of the swine and rat studies using corn oil as a vehicle will provide a basis for comparison of results across species.

Methods and Materials

Soil Selection

In preparation for the pilot study, six candidate test soils were collected by CH2M Hill in June 2004. The soils were collected as described in the *Sampling and Analysis Plan – Soil Sampling for the Pilot Bioavailability Study* (provided in Appendix A). These soil samples (approximately 3 gallons each) were shipped to Exponent's Boulder, Colorado, laboratory, where they were air-dried and homogenized, and approximately 500 g was sieved to <250 μm (60 mesh). A 50-g aliquot of each sieved sample was then shipped to Alta Analytical Laboratory (Alta) in El Dorado Hills, California for analysis of polychlorinated dibenzo-*p*-dioxin and furans (PCDD/Fs) by high-resolution gas chromatography/mass spectrometry (HR-GC/MS; EPA Method 8290). Results from these analyses are presented in Table 1. Neither of the Midland soils (TCDD concentrations of 15.2 and 59.5 pg/g TCDD, respectively; Table 1) had TCDD concentrations as high as those in a soil that had been collected previously in bulk from Midland (CC-S-27, which contains 163 pg/g TCDD [Table 1] as reported in Exponent 2003; collected from the southeast portion of the Dow Corporate Center lawn in May 2002 and archived dry in Exponent's Boulder laboratory). Because the CC-S-27 soil exhibits a congener profile consistent with Midland soils (TEQ dominated by TCDD and 1-PeCDD) this soil was selected for the pilot study. Sample THT02769 (from location Imerman Park 2) was selected as the Tittabawassee River soil for use in the pilot study, because it exhibited a congener profile consistent with the flood plain sediments (TEQ dominated by 4-PeCDF and TCDF) and had a total TEQ concentration close to 1,000 pg/g (Table 1).

The remainder of soil THT02769 was sieved to <250 μm, and the entire sieved soil mass was homogenized. Triplicate splits of soils CC-S-27 and THT02769 (collected using a soil splitter, as were all soil aliquots used in this study) were sent to Alta to test for homogeneity of the soil batches. Results from these analyses are presented in Table 2. Coefficients of variability (CVs) for the five congeners that contribute the most to total TEQ in soil CC-S-27 ranged from 1.9% to 5.6% for the triplicate analysis. CVs for the triplicate analysis of soil THT02769 ranged from 16.1% to 19.7%, and resulted from one of the triplicate samples contributing greater concentrations of PCDD/Fs than the other two (Table 2). Soil THT02769 was subsequently rehomogenized and used for the study. Co-planar PCB concentrations in each of the two study soils were also analyzed in one of the triplicate samples (EPA Method 1668); these data are also presented in Table 2.

Methods used to perform the pilot bioavailability study are described in the document titled, *Pilot Study Design: Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils* (provided in Appendix B).

Dose Preparation and Administration

Rat Study

Each of the test soils (<250-μm size fraction) was blended with PMI Nutrition International, Rodent LabDiet® 5001 (meal) (5% w/w) at WIL Research Laboratories, Inc. (WIL) in Ashland, Ohio. The WIL report describing the diet blending is provided in Appendix C, and results for PCDD/Fs in the Rodent LabDiet® batches used in this study are provided in Table 3. To accomplish the blending of soil into the rat diet, soil (475 g) and diet (1,000 g) were blended in a Hobart mixer for 5 minutes to create a diet pre-mixture. The pre-mixture was then blended with 8,025 g of diet in a V-blender to create the final 9,500-g diet batch. Diet homogeneity samples (25 g) were collected from the initial, middle, and final material that emerged from the V-blender; these samples (three samples per blended diet) were sent to Alta for analysis of PCDD/F concentrations. Results for the pre-dosing soil/diet mixtures (Table 4) indicate that for the CC-S-27/diet blend (Test Article #1), the five congeners that contributed most greatly to TEQ were recovered at 79%–131% of expected concentrations (based on concentrations measured in the test soil), and CVs for the pre-dosing triplicate analyses ranged from 2.3% to 12%. For the THT02769/diet blend (Test Article #2), the five most important congeners were recovered at 76%–100% of expected concentrations, with CVs ranging from 4.5% to 14%. These measurements of blended diet PCDD/F concentrations and homogeneity were considered acceptable to proceed with the study.

For the reference material in diet (matched to soil CC-S-27), TCDD, 1-PeCDD, 1,6-HxCDD, 1,4,6-HpCDD, and 4-PcCDF (the five dioxin/furan congeners contributing most greatly to TEQ for this soil) were spiked into 200 mL acetone (B&J Brand®, High Purity Solvent; previously analyzed for dioxins/furans and determined to be below detection limits for all congeners) at concentrations that, once blended with feed, would deliver the same dose of these five congeners as the CC-S-27/diet blend. Analytical results for the reference mixture in acetone are provided in Table 5. At WIL, the acetone (100 mL) and diet (1,000 g) were blended in a Hobart mixer for 5 minutes to create a diet pre-mixture. The pre-mixture was then blended with 8,500 g of diet in a V-blender to create the final 9,500 g diet batch (Test Article #3). Diet homogeneity samples (25 g) were collected from the initial, middle, and final material that emerged from the V-blender; these samples were sent to Alta for analysis of PCDD/F concentrations (Table 4). For Test Article #3, the five spiked congeners were recovered at 83%–118% of expected concentrations in the pre-dosing diet samples, with CVs ranging from 1.0% to 3.0%. Based on these results, the concentrations and homogeneity of PCDD/Fs in Test Article #3 were considered acceptable to proceed with the study.

The two gavage reference materials for the rat study were prepared in corn oil/acetone (99:1), and were designed to deliver the same dioxin/furan doses as the soil/diet blends. To create these reference mixtures, the five dioxin/furan congeners that contribute most greatly to TEQ in each soil were spiked into acetone (20 mL), and the concentrations of the five congeners in the spiked acetone was measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 8.26 mL of this acetone was added to 817.7 mL corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations [Table 3]). The two corn oil/acetone

reference materials were then assayed for concentrations of the five target congeners (Table 5). Relative percent differences (RPDs) between target and pre-dosing measured concentrations were generally in the range of 3%–13%, except for 1,2,3,6,7,8-HxCDD, which was present at a concentration approximately 40% greater than the target concentration. Because this compound contributed less than 5% of the total TEQ of the soil and reference oils, this variation was considered acceptable for use in the study. The gavage reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

Swine Study

For the swine pilot study, the test-soil doses were delivered by placing 1 g of the soil (either CC-S-27 or THT02769 in the center of a 10-g moistened dough ball (Zeigler Bros. Swine Diet) and offering it to the swine. The swine were fasted for two hours prior to dosing, because previous studies conducted in this animal model have indicated that a 2-hour fast will ensure eager acceptance of the 10-g dough ball containing the dose. Soil-containing dough balls were prepared every 3–4 days. Five dough balls (containing a total of 5 g of test soil) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 10 g soil/day. Immediately after dosing, the animals were given one-half of their standard ration of swine feed. The two dose groups receiving the soil doses (Groups 3 and 4) had their feed rations reduced by 80 g/day to compensate for the greater number of feed balls given these animals during dosing, relative to the corn oil–dosed animals. Dosing and feeding continued twice daily for 30 consecutive days.

The dosing materials for the two reference groups were prepared in corn oil/acetone (99:1), and were designed such that 2 mL of the corn oil/acetone mixture would deliver an equivalent dose to 5 g of the test soil to which it was matched. To create these reference mixtures, the five dioxin/furan congeners that contribute most greatly to TEQ in each soil were spiked into acetone (20 mL), and the concentrations of the five congeners in the spiked acetone were measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 10 mL of this acetone was added to 990 mL corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations [Table 3]). The two corn oil/acetone reference materials were then assayed for concentrations of the five target congeners (Table 6). Relative percent differences (RPDs) between target and measured concentrations were in the range of 1%–21%, which was considered acceptable for use in the study. The swine reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

For dosing, 1 mL of corn oil/acetone mixture was placed in each gel capsule (Torpac, 1.2 mL volume), and these were embedded in the center of a 10-g ball of moistened swine feed. The oil-filled gel capsules were inserted in dough balls immediately prior to dosing. Two dough balls (containing a total of 2 mL of reference mixture) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 4 mL reference mixture/day. Immediately after dosing, the animals were given one-half of their standard ration of swine feed. Dosing and feeding continued twice daily for 30 consecutive days.

Animal Handling and Dosing

Rat Study

Animal handling and dosing during the rat study were performed as described in the pilot study design document (see Appendix B), a brief summary of which follows.

Fifty 4-month-old female Sprague-Dawley rats, weighing between 210 and 240 g, were obtained from Harlan (Indianapolis, Indiana) and placed in individual stainless steel cages. Each rat was weighed on arrival (Day –6), then on Day –2 (during the quarantine period) and Day 3 of the dosing period, and then weekly until study termination. The rats were provided with PMI Nutrition International Rodent LabDiet® 5001 (meal) and de-ionized water *ad libitum* during the one-week quarantine period, and their health status was monitored. All LabDiet® 5001 fed to the rats (including during the quarantine period and to the gavage dose groups during the dosing period) was from the same two batches of LabDiet® 5001 that were used by WIL Research to prepare the blended rat diets (Table 3). Two days prior to the start of dosing, healthy animals were randomly assigned to five dose groups (10 rats/group; dose groups are identified in Table 7).

During the 30-day dosing period, each rat received 50 g of feed every 2 days (background feed for Groups 1 and 2, and dosed feed for Groups 3, 4, and 5). The weight of any unconsumed feed at the end of each 2-day period was measured, and an estimate was made of the weight of any spilled feed. Dose groups 1 and 2 were gavaged daily at 11 a.m. with 1 mL of the corn oil/acetone reference mixtures.

Twenty-four hours after the last dose was administered, the rats were weighed and terminated under CO_2 anesthesia. Their livers were excised, blotted dry, weighed and wrapped in foil. The liver samples for the ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-deethylase (MROD) assays were collected (1-g samples) from the livers of each pair of rats (i.e., 0.5 g collected from each individual liver). The sample was minced, placed in a 2-mL cryovial, immediately frozen in liquid nitrogen, and sent to Michigan State University (MSU) for analysis. The remainder of the pair of livers was then frozen and shipped to Alta, where they were homogenized together to create a sample of sufficient mass for the analytical work. As much fatty tissue as possible (3–6 g) was collected from within the abdominal cavity of each rat, weighed, and wrapped in foil. The fat samples were frozen and shipped to Alta, where the fat samples from each pair of rats were homogenized together to create a sample of sufficient mass for the analytical work.

Triplicate 25-g post-dosing subsamples of each blended rodent diet were collected and shipped to Alta for analysis of dioxins/furans, to evaluate the stability of the blended diets during the 30-day dosing period, and to confirm the doses of dioxins/furans delivered to the rats (Table 4). The CV between all six samples of the blended rodent diet (three pre-dosing and three post-dosing) was no greater than 22% for any congener, indicating that the diets were stable during the study. In addition, the gavage reference mixtures were shipped to Alta for post-dosing analysis (Table 5). The CV between the pre- and post-dosing gavage reference mixtures was no

greater than 21%, indicating that the reference mixtures were also stable during the study period.

Only two rats, both from Group 2, did not complete the 30-day dosing period. Rats #29 and #24 were sacrificed after 15 and 20 days of dosing, respectively, due to persistent problems with administering the gavage dose. On necropsy, it appeared that there was a stricture immediately prior to the stomach of the first rat, and it was found that the esophagus of the second rat had been perforated.

Rat carcasses from the pilot study were wrapped in foil, placed in individual labeled zipper-sealed freezer bags, and archived (–80 °C) for possible further analysis.

Swine Study

Animal handling and dosing during the swine study were performed as described in the pilot study design document (see Appendix B), a brief summary of which follows.

Twenty intact male swine weighing between 8.4 and 10.7 kg were obtained from Chinn Farms (Clarence, Mississippi) and were fed a specially formulated diet (Ziegler Bros. Inc., Gardners, Pennsylvania). Swine were weighed on arrival (Day –8), on Days –4 and –1 during the quarantine week, and then every three days until study termination. Feed was given at 4% of body weight per day, and was adjusted every three days to maintain a constant feed rate during the study. The swine were housed in stainless steel cages, and their health status was monitored during the 1-week quarantine period. Two days prior to the start of dosing, healthy animals were randomly assigned to four dose groups (five swine/group; dose groups described in Table 8).

Three swine were culled prior to the start of the dosing period (e.g., 23 animals were obtained from Chinn Farms, but only 20 were dosed during the study), and these animals were maintained on the weighing/feeding schedule described above, but were not given any doses. At the end of the study, these three animals were necropsied, and body composition of skin, fat, and muscle, as a proportion of body weight, was determined for each animal.

All doses were delivered twice daily in purified feed dough balls, as described in the dose administration section, at 9:00 a.m. (immediately prior to the morning feeding) and at 4:00 p.m. (immediately prior to the afternoon feeding) for 30 days. Twelve hours after the final dose, the animals were weighed and humanely sacrificed, and liver and fat samples were collected for analysis.

Only one animal, from Group 4, did not complete the 30-day dosing period. This animal was found dead in his pen on the morning of the 25th day of the study (he had been ill with what appeared to be a systemic infection, and had been given the antibiotic Naxcel [sodium ceftiofur] for the 9 days prior to his death).

The whole liver of each animal was excised, blotted dry, and weighed. Three 1-gram samples were collected for EROD and MROD assays (for each sample, subsamples from three sections of the liver were collected and diced), placed in 5-mL cryovials, and immediately frozen in

liquid nitrogen. These samples were shipped in liquid nitrogen to MSU for EROD/MROD analysis. The remainder of the liver was wrapped in foil, placed in a zipper-sealed freezer bag, and frozen at –80 °C. Fatty tissue from the abdominal wall, plus a small amount from the abdominal cavity (40–65 g, total) was collected, wrapped in foil, and frozen at –80 °C. The liver and fat were shipped (frozen) to Alta. The residual reference mixtures were shipped to Alta for analysis. The CV between the pre- and post-dosing reference mixtures ranged from 9% to 28%, indicating that the reference mixtures were stable during the study period (Table 6).

All swine carcasses were were double-bagged in heavy black plastic trash bags and stored at –20 °C, in case additional samples were needed.

Tissue Sample Homogenization and Analysis

At MSU, liver microsomes were prepared from each liver sample, and the protein levels and enzymatic activities were measured according to the MSU Standard Operating Procedure (SOP) No. 250 (v 1.1), titled *Protocol for Liver Microsome Preparation, and Microsomal Protein Measurement and AROD Assays in the same 96-Well Plate*. EROD/MROD activities and protein concentrations were measured fluorometrically at the end of the assay, using a Cytofluor multiplate reader.

At Alta, the rat liver samples were homogenized using a Cuisinart mini-prep processor. The processor was run on the "high" setting until the sample was liquefied (for the liver samples) or thoroughly homogenized (for the fat samples). The sample was then poured into separate 40-mL amber glass VOA vials for extraction. After homogenization of each sample, all parts of the processor that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then rinsed with ultra-high-purity solvents (hexane followed by dichloromethane).

The swine liver samples were homogenized using a Villaware model 5265-05 power grinder. The grinder was fitted with a 4-mm-diameter mesh gate for all grinding. Samples were collected directly from the grinder into labeled amber glass jars. Between samples, all parts of the grinder that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity solvents (acetone, toluene, hexane, and dichloromethane).

The rat and swine fat samples were homogenized with a Sumeet Multi-Grind Model 964, a small-volume grinder suitable for small sample sizes. Samples were collected directly from the grinder into labeled amber glass jars. Between samples, all stainless steel parts of the grinder that were in contact with sample material were washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity solvents (acetone, toluene, hexane, and dichloromethane). The polycarbonate grinder lid was washed with soap and hot water, rinsed with de-ionized water, and then serially rinsed with ultra-high-purity methanol followed by hexane.

Subsamples of the liver and fat homogenates were extracted in methylene chloride/hexane and analyzed for lipid content (EPA Method 1613), and PCDD/F concentrations by HR-GC/MS

(EPA Method 1613). Selected samples were also analyzed for co-planar PCBs (EPA Method 1668).

Estimation of Relative Bioavailability

Relative bioavailability was estimated by comparing the fraction of administered dose retained in the tissues of animals in the groups dosed with soil with the fraction of administered dose retained by animals given the reference vehicle(s) (oil or feed), similar to the method used by Wittsiepe et al. (2004). Several assumptions were made in this estimation process:

1. *The whole-body elimination rate for each compound would be the same in the reference-dosed animals as in the soil-dosed animals, and can be approximated by a first-order model.* Diliberto et al. (2001) demonstrated that, in mice exposed subchronically to TCDD, the fraction of administered dose retained in the animal tissues decreased as the body burden increased, indicating an increase in elimination rate with increasing body burden. To account for this issue, reference dosing materials for each group were formulated to try to match the anticipated administered soil doses for that group. In addition, measurements of hepatic EROD and MROD activity were made for each group to assess whether enzyme induction (and the associated increase in hepatic metabolism) was occurring, and if so, whether it was occurring to a different extent in soil-dosed groups than in reference groups. EROD activity is a marker for the CYP1A1 enzyme, while MROD activity is a marker for CYP1A2 activity. CYP1A1 is the enzyme that mediates metabolism of several PCDD/F compounds, while the CYP1A2 protein in the liver serves as a binding protein for many PCDD/F compounds. When CYP1A2 is induced, hepatic sequestration of these compounds occurs. For some compounds, this hepatic sequestration may result in either a greater or lesser elimination rate, depending on the compound, its binding affinity for CYP1A2, and the mechanism of metabolism. If either enzyme is induced to a different extent in the soil-group animals compared to the reference-group animals, the assumption of equivalent whole-body elimination rates between groups would likely be violated.

2. *The majority of retained administered dose would be distributed in liver and adipose tissues, and the proportion of retained dose distributed to* ***tissues other than liver and adipose*** *would not be different in soil-dosed groups compared to reference-dosed groups.* Distribution studies following subchronic administration of TCDD in mice and rats demonstrate that, at the lowest doses tested, liver and adipose account for 70% to 80% of retained body burden; this percentage increases to approximately 90% at higher tested doses (Diliberto et al. 2001; Hurst et al. 2000). The remainder of the retained compound in these studies was found in skin and muscle, and concentrations were consistent with simple lipid-based partitioning of compound in these tissues.

The relative bioavailability (RBA) of a compound from soil administration, compared to administration of a reference material ($RBA_{soil:ref}$), is the ratio of the absolute absorption fractions (f_{abs}) of the compound from the two media:

$$RBA_{soil:ref} = \frac{f_{abs,soil}}{f_{abs,ref}}$$

In general, after daily administration of a compound, the amount of compound in the body at the end of 30 days is a function of both the administered dose rate and the elimination rate. Using the assumption of first-order elimination, the whole-body amount of compound as a function of time can be estimated as follows:

$$Q_{body} = \frac{D * f_{abs}}{k}(1 - e^{-kt})$$

where:

Q_{body} = mass of compound in body, ng

D = daily administered dose, ng/d

k = elimination rate, d^{-1}

t = duration of dosing, d

Solving for f_{abs},

$$f_{abs} = \frac{Q_{body}\ k}{D\,(1 - e^{-kt})}$$

Solving for the RBA,

$$RBA = \frac{Q_{body,soil}\,k \,/\, D_{soil}\,(1 - e^{-kt})}{Q_{body,ref}\,k \,/\, D_{ref}\,(1 - e^{-kt})}$$

Because the elimination rate, k, is assumed to be equal between the two groups, and because the time of administration, t, is the same, this simplifies to:

$$RBA = \frac{Q_{body,soil} \,/\, D_{soil}}{Q_{body,ref} \,/\, D_{ref}}$$

Again, the time of administration is the same for both groups, 30 days, so the daily doses for the two groups can be converted to the total administered dose:

$$RBA = \frac{Q_{body,soil} / Q_{ad\,\min,soil}}{Q_{body,ref} / Q_{ad\,\min,ref}}$$

where:

Q_{admin} = total mass of compound administered

The ratio of Q_{body}/Q_{admin} for a given dose group is the fraction of administered dose retained in the body (*FR*). Thus, the RBA evaluation for soil compared to a reference group simplifies to:

$$RBA = \frac{FR_{soil}}{FR_{ref}}$$

As discussed above in assumption 2, distribution studies for dioxin demonstrate that liver and adipose tissue account for the majority of dioxin retained in the body (70% to 90%, depending on the species and dose range tested; Diliberto et al. 2001; Hurst et al. 2000). Thus,

$$Q_{body} = Q_{liver} + Q_{adipose} + Q_{other}$$

where Q_{tissue} is the product of the concentration of compound in the tissue, C_{tissue}, and the weight of the tissue, w_{tissue}. Then, the fraction of administered dose retained in a given tissue is:

$$FR_{tissue} = \frac{Q_{tissue}}{Q_{ad\,\min}}$$

If the proportional distribution of compound among tissues is the same among dose groups, then an RBA value can be calculated on the basis of a single tissue or on the basis of a combination of tissues. As discussed above, for this effort, liver and adipose tissues serve as the basis for the RBA calculation. Liver weights were measured at sacrifice for rats and swine. Adipose tissue weights for the rats were estimated as a function of body weight at sacrifice using the relationship from Brown et al. (1997) based on data for male Sprague-Dawley rats developed by Bailey et al. (1980; as cited by Brown et al. 1997).

$$w_a = (0.0199*BW + 1.644) / 100$$

Adipose tissue weights for the swine were estimated as a percentage of body weight using the results of the total fat dissection for the three control swine described above.

Results

Rat Study

As discussed in the Animal Handling and Dosing section, two rats from the Tittabawassee River gavage oil reference group (Group 2) were sacrificed before the end of the study (after 15 and 20 days of dosing) due to persistent problems with administering the gavage dose. Results from this rat pair were not included in the data analysis discussed below.

Feed Intake

Details of feed intake for all groups are presented in Table D-1, and the feed intake is illustrated in Figure 1. The mean daily feed intake for all dosing groups was approximately 16 g/day. The mean daily intakes for the two oil reference groups were 14 g/day and 13 g/day, for the Midland oil and Tittabawassee River oil reference groups, respectively. The mean daily feed intake for the Midland soil group was 17 g/day (Group 3) and 19 g/day for the Tittabawassee River soil group. The mean daily feed intake for the Midland feed reference group (Group 5) was 16 g/day. The lower feed consumption in the oil gavage groups compared to the soil/feed and reference feed groups is consistent with the expectation that these groups might consume less feed due to caloric intake from the oil gavage vehicle (9 kcal per g, or about 8 kcal per mL; USDA National Nutrient Database for Standard Reference, Release 17, 2004). This is approximately 15% of the caloric intake from feed observed in the soil groups, so the lower feed intake in the oil gavage groups is consistent with an adjustment of feed intake by the animals, reflecting the caloric intake from corn oil gavage.

The doses and reference materials had been prepared assuming that the rats would consume 23 g/day, based on a literature value (Freeman et al. 1992), so the observed daily feed intake was less than anticipated. The feed was administered in a loose meal form rather than pellets, and this may have influenced feed intake rates. This lower feed consumption resulted in the administered doses of study compounds for the gavage oil groups being higher than the soil groups (see below in Administered Dose section).

Body and Liver Weights

Rat body weights for all five dosing groups averaged 238 g at study initiation (study day –2), and 259 g at study termination (Figure 2; detailed data for all animals are presented in Table D-2), a gain of 9% over the 30-day study period. This weight gain was similar to the 10% gain observed in the background study, and reflects the fact that female Sprague-Dawley rats have already reached adult body weight at 4 months of age. Rat liver weights at study termination ranged from 7.3 to 11.4 g (average of 9.0 g) over all dosing groups, approximately 3.5% of body weight (Table D-3).

Administered Doses

The average daily doses of contaminants in each group are summarized in Table 9. Doses received by the rats in the oil and feed reference groups were generally somewhat higher than the doses received in the soil group. This is due primarily to two factors: lower feed consumption rates for the soil/feed-dosed animals than expected based on literature values, and deviations from the targeted concentrations in both the soil/feed mixture and in the reference materials. The literature-based feed consumption values were used to establish the target corn oil concentrations.

EROD and MROD Activity

Mean EROD and MROD activities in rat liver tissue from all dose groups are reported in Table 10, and the complete data set is presented in Table D-4. EROD activity was statistically significantly elevated in both reference material groups compared to the paired soil groups. MROD activity was elevated in reference groups compared to soil groups, but the difference was not statistically significant. This result is consistent with the difference in dosing rates between the reference and soil groups, and indicates that the dosing rates in the reference groups were sufficiently greater than the soil groups to result in increased enzyme induction.

RBA Estimates

Concentrations of contaminants in liver and adipose tissues from each pair of rats are reported in Tables D-5 and D-6. Tissue concentrations of the contaminants of interest were all above detection limits for all dose groups and compounds and were also greater than the instrument calibration limits in nearly all samples (Table 11). Figure 3 illustrates the fraction of administered dose present in liver and adipose tissues, and in the summed tissues, for all dose groups. A larger proportion of administered dose was retained in liver than in adipose tissue for all dose groups. The coefficient of variability was generally in the range of 10% to 15%, with one exception (Table 12). In the Tittabawassee River flood plain soil group, the liver concentration in one rat pair of 1,2,3,6,7,8-HxCDD was approximately four times greater than the concentrations in the other rats in this group, and corresponded to a retained dose in liver greater than the total administered dose of this compound. The adipose tissue concentration for this rat pair was not significantly different from the others in the group. This data point qualifies as an outlier at the 1% level using Dixon's extreme value test, and was omitted from further calculations of relative bioavailability.

Estimates of average relative bioavailability of the two soils in rats, based on comparisons of fraction of dose retained in liver, adipose, or the sum of liver and adipose tissues in reference materials, are presented in Table 12 and Figure 4 (calculated as described in the section on Estimation of Relative Bioavailability). For the Midland soil, comparison to the reference feed produces higher relative bioavailability estimates than comparison to the reference oil gavage. This is expected due to the lower absolute bioavailability of contaminants from feed compared to corn oil.

The relative bioavailability of the feed reference mixture compared to the corn oil reference mixture for the Midland soil congener pattern is shown in Figure 5. As expected, congeners in feed were somewhat less bioavailable than congeners in the reference corn oil, with RBA (reference feed compared to reference oil) ranging from about 60% to 80%.

Swine Study

One animal from the Tittabawassee River soil group (Group 4) became ill during the study and was found dead on day 25. Results from this animal were not included in the data analysis discussed below.

Body and Liver Weights

Swine weights for all dosing groups averaged 11.3 kg at study initiation (Study Day –1), and 28.0 kg at study termination (Figure 6; see Table D-7 for detailed individual animal data), a gain of 149% over the 30-day maintenance on the Ziegler Bros. swine diet. This rapid weight gain is typical of juvenile swine. For each dosing group, the initial group mean body weights ranged from 10.8 kg to 11.7 kg, and at study termination, group mean body weights ranged from 27.2 kg to 28.6 kg. The group mean weight gains ranged from 145% to 155%, with consistent weight gains for all four groups throughout the 30-day study. Swine liver weights for all four groups ranged from 501 to 796 g (average of 653 g, or 2.3% of bodyweight). The group mean liver weights ranged from 585 g to 731 g (Table D-8).

Swine Necropsy and Body Fat Dissection Results

As described earlier, three additional swine were maintained on the weighing and feeding schedule, but were not dosed. These three swine were analyzed to determine the body composition of muscle, skin, and fat as a percentage of body weight (Table D-9). The percent of body weight that was muscle ranged from 52.9% to 57.6% (average 55.2%), and the percent of body that was skin ranged from 7.25% to 7.50% (average 7.41%). The body fat as a percent of body weight ranged from 6.22% to 7.22%, with an average of 6.74%. This average value was used to determine the weight of adipose tissue based on body weight in the RBA calculations.

Administered Doses

The average daily doses over the 30-day study for all swine study groups are summarized in Table 13. The administered dose for the reference oil groups matched those for the soil groups much more closely than in the rat study. This is due primarily to the mode of administration of soils in the swine study, in which weighed amounts of soil were wrapped in dough balls and fed directly to the swine, rather than mixed with loose feed material. Administered doses on a ng/kg bw/day basis were much lower than in the rat study, due to the larger animal size and limitations in how much soil can be effectively administered to the animals.

EROD and MROD Activity

Mean EROD and MROD activities in swine liver tissue from all dose groups are reported in Table 10, and the complete data set is presented in Table D-10. In contrast to the rat study, no statistically significant differences in EROD or MROD activity between soil and corresponding reference oil groups were observed. This is consistent with the better matching of doses between soil and reference oil groups in the swine study compared to the rat study.

RBA Estimates

Concentrations of contaminants in liver and adipose tissues from each animal are reported in Tables D-11 and D-12. In contrast to the rat study, tissue concentrations of the contaminants of interest did not always exceed the limits of detection, particularly for the Midland soil group. Table 14 summarizes the numbers of non-detected results per tissue and dose groups for the swine study. The prevalence of non-detected results in the swine studies necessitates consideration of appropriate handling of non-detects in the analysis of the data. Dual data analyses were conducted for all swine data, assuming either one-half the detection limit or the detection limit for all non-detects in the data set. There were also a number of results that were below the lower calibration limit of the lab equipment (qualified with a "J"). These were identified and handled as detected values with the reported concentrations used in calculations.

Figure 7 illustrates the fraction of administered dose present in liver and adipose tissues, and in the summed tissues, for all dose groups, assuming either one-half the detection limit or the detection limit for all non-detected results. The fraction of administered dose retained in adipose is greater than in liver in the swine, in contrast to the pattern observed in rats. The inter-animal variability in tissue concentrations and fractions retained is greater in the Midland soil and corresponding oil reference group compared to the Tittabawassee River flood plain groups. This is consistent with the lower doses in the Midland soil groups, which resulted in tissue concentrations near or below the detection limits in many cases, resulting in greater variability. However, the variability among animals in the Tittabawassee River flood plain soil group and corresponding oil reference group was comparable to the variability observed in the rat data.

Estimates of average relative bioavailability of the two soils in swine based on comparisons of fraction of dose retained in liver, adipose, or the sum of liver and adipose tissues in reference materials, are presented in Tables 15a and 15b and Figure 8. The RBA values across tissues are generally consistent with one another. No reliable RBA values for 1-PeCDF and TCDF for the Tittabawassee River flood plain soil using liver tissue only could be calculated. Liver tissue concentrations for these compounds were undetectable in all of the soil group animals. In addition, in the corn oil reference group, 1-PeCDF was undetectable for four of the five liver samples, and below the instrument calibration limit in the fifth sample. Given the lack of detectable liver concentrations in the soil group for these compounds, RBA estimates based on swine ***liver*** tissue for these two compounds cannot be made. The RBA estimates for these compounds based on adipose tissue are based on detectable results, and the combined fraction retained in liver and adipose tissue is dominated by the adipose tissue results, so the RBAs based on adipose tissue and the combined tissue are reliable.

Discussion

Sensitivity of Models

Tissue concentrations achieved in rats after 30 days of administration of soils and reference compounds were consistently above analytical detection limits for both liver and adipose tissue (Table 11). In contrast, in swine dosed with the Midland soil, a substantial fraction of both adipose tissue and liver samples displayed specific congener concentrations below detection or analytical lower calibration limits. In swine dosed with Tittabawassee River flood plain soil, adipose tissue levels were generally detectable. In liver tissue, TCDF and 1-PeCDF were not detected in any of the soil group animals, but the remaining compounds were generally detectable in swine liver (Table 14).

For animal tissues and compounds in which the analytes were generally detectable, the results were generally consistent from one animal (or pairs of animals, in the case of the rats) to another, resulting in coefficients of variation (CVs) on the estimated mean RBA values in the range of 10% to 25% (Tables 12 and 15). The CVs were larger for specific congeners in the swine study of Midland soil for which a substantial number of non-detects were obtained. The use of fraction of dose retained in liver plus adipose tissue as the basis for the RBA calculations produced generally stable results, although, as discussed further below, the rats and swine showed different patterns of distribution between liver and adipose tissue. Increasing the number of animals per dose group might decrease the CVs observed, but the variation observed in this study is probably sufficiently small to be acceptable.

Consistency of Models

Distribution Patterns

The retention and distribution of test compounds between liver and adipose tissues in the rats and swine are summarized in Figure 3 and 7. In general, rats retained higher percentages of the total administered dose at the end of 30 days than did swine for both soils. Swine exhibited modest liver sequestration for most compounds, compared to substantial liver sequestration for most of the tested compounds in rats (Figure 9). This may reflect, in part, physiological differences between swine and rats, or it may be a result of the lower liver tissue concentrations resulting from the lower administered dose and large swine growth rate compared to the rats. At the higher dose rates used in the rat study, the relatively high hepatic retention compared to adipose tissue suggests that some induction of CYP1A2 protein is likely occurring in all groups, even though differences in MROD activity between groups were not significant. CYP1A2 protein in liver binds several of the PCDD/PCDF compounds effectively, resulting in hepatic sequestration. In the swine, lower doses on a body-weight basis were used, resulting in lower hepatic TEQ concentrations. The concentrations in swine tissue may be low enough that substantial induction of CYP1A2 protein did not occur, and thus, less marked hepatic sequestration occurred.

RBA Estimates

The RBA estimates obtained in swine were statistically significantly lower than those obtained in rats for all of the congeners tested in the Tittabawassee River flood plain soil and for TCDD in the Midland soil (Figures 10 and 11). In contrast, the RBA obtained in swine for 1,2,3,4,6,7,8-HpCDD in the Midland soil was statistically significantly higher than in rats (mean RBA estimates of 0.55 in swine and 0.34 in rats, $p<0.05$). The EROD and MROD enzyme activity data may shed light on some of these differences. The EROD data suggest differential enzyme induction in the rats between the reference and soil groups for both soils, with significantly greater EROD activity in the reference groups compared to the soil groups (Table 10). As discussed above, EROD activity is a marker for induction of CYP1A1. CYP1A1 is responsible for the metabolism of 2,3,7,8-TCDF in rats (Tai et al. 1993), and induction of CYP1A1 has been shown to strongly increase the hepatic metabolism rate for TCDF in rats (McKinley et al. 1993; Olson et al. 1994). 4-PeCDF also can induce its own metabolism due to induction of CYP1A enzymes (Brewster and Birnbaum 1987). Other compounds, including TCDD and 1-PeCDF, show decreased retention of administered dose with increasing dose in subchronic studies, suggesting autoinduction of metabolism, although the specific metabolic pathways have not been identified (DeVito et al. 1998; Diliberto et al. 2001; Jackson et al. 1998). The metabolic pathways for the other compounds that contribute substantially to the total TEQ in the Midland and Tittabawassee River flood plain soils have not been examined to date, but may be influenced by CYP1A1 induction.

The statistically significant increase in EROD activity in rats treated with the reference corn oil and reference feed materials corresponds to the increased doses of these compounds received by the reference groups compared to the soil groups. This was due to lower-than-targeted concentrations of key contaminants in the soil/feed mixtures, as well as lower feed intake in the soil/feed rat groups than estimated prior to the experiment (although growth and body weight were not affected), resulting in lower administered dose in the rat soil groups than initially targeted (Table 9). In addition, if the relative bioavailability of the TCDF or other congeners in soil was low, the actual differential in absorbed dose of furan compounds between the two groups may have been much higher. The RBA estimates developed in swine for the Tittabawassee River flood plain soil PCDF congeners indicate that these congeners were approximately one-fourth as bioavailable as in corn oil. This indicates that, even if the administered doses of compounds in the soils and reference corn oil mixtures were equal, the absorbed doses may have differed by nearly a factor of four.

Increased EROD activity in reference-group rats compared to soil-group rats could result in an increase in hepatic metabolism rates in the reference-group rats, especially for TCDF. Such a differential in metabolism rates would violate the assumption (discussed above in the methods section) that rates of elimination in the soil and reference groups are the same. A greater elimination rate in the reference groups compared to the soil groups would result in an apparently greater relative bioavailability for the soil group. That is, a larger percentage of the *absorbed* dose would be retained in the soil groups compared to the induced reference groups that would be eliminating absorbed compound more rapidly. Thus, the high relative bioavailability estimate obtained in rats for TCDF in the Tittabawassee River flood plain soil may be in part due to elevated elimination rates in the reference groups, consistent with the elevated EROD activity observed in these groups. The statistically significant increase in

EROD activity in reference-group rats compared to soil-group rats may have resulted in higher metabolic rates in the reference-group rats for compounds of interest other than TCDF as well.

In contrast with the rats, the swine did not exhibit a statistically significant difference in EROD activity between the soil and reference material groups (Table 10). This is consistent with the better control of soil dosing rates in this model and could account for at least some of the apparent inconsistency in estimated relative bioavailability between the rats and swine in this study.

The EROD and MROD activities for all of the animals in the study are plotted in Figure 12. For rats, EROD activity is strongly correlated with hepatic TEQ, while MROD shows a weaker relationship. In swine, EROD and MROD activity are also correlated with hepatic TEQ, but MROD shows a stronger relationship. The positive dose-response for EROD and MROD, even at the low doses used in these studies, indicates that in future studies, in order to avoid differential EROD and MROD induction and activity among groups, soil and reference administered doses will need to be matched more closely. In fact, administered doses should probably be adjusted to reflect expected differences in relative bioavailability. That is, if the relative bioavailability is expected to be in the range of 25% to 75 percent for soil compared to reference corn oil materials, the administered dose of compounds in the reference corn oil material could be reduced by 25% to 50% compared to the soil dose, to try to ensure similar absorbed doses between the two groups. This approach should minimize any differences in enzyme induction between soil and reference groups.

Comparative Evaluation of Rat and Swine Models

For reasons of efficiency in a full bioavailability study of a number of soils, it would be desirable to identify a single animal model, rather than continue with two animal models. Swine are the preferred animal model for humans in research on the bioavailability of lead and arsenic from soils for a variety of biological reasons (Weis and Lavelle 1991). Wittsiepe et al. (2004) used minipigs in an evaluation of PCDD/F bioavailability from soils based on an evaluation of their gastrointestinal tract similarity to humans (Swindle and Smith 1998). Young pigs have comparable physiology and have been used successfully as a model for gastrointestinal function of children (Dodds 1982; Miller and Ullrey 1987). However, evaluation of swine as a model for humans in the study of highly lipophilic compounds is much less complete. Kararli (1995) notes that for highly lipophilic compounds, bile fluid plays an important role in absorption and uptake. Rats have no gallbladder, so the patterns of secretion of bile fluid are different from those in animals that do have gallbladders (including humans and pigs). However, there is a lack of comparative studies among swine, rats, and humans for assessing the bioavailability of lipophilic compounds, so there is no clear reason to prefer swine over rats as a model for human bioavilability of PCDD/Fs from soil.

From a practical perspective, additional issues could influence the choice of a single animal model. Arguments in favor of the rat model include:

- In this pilot study, rats were more sensitive than swine based on tissue detection limits, due to the ability to administer a larger dose of soil on a

body-weight basis and smaller relative changes in body weight over the course of the study. The swine dosing regimen would need to be altered to improve the sensitivity of this model for soils with contaminant concentrations in the same range as or lower than the Midland soil tested here.

- The swine growth rate was very large, with body weights more than doubling over the course of the 30-day experiment. In contrast, rat body weights were more consistent. The rapid growth of the swine decreases the sensitivity of the model, because the volume of distribution for the administered compounds more than doubles over the course of the study.

Arguments in favor of the swine model include the following. Control of soil dosing levels was easier to achieve in swine because of the method of administration. For swine, a measured amount of soil was wrapped in a dough ball and fed directly to the animal. For the rats, soil was mixed with rat feed (in a meal form) at the maximum proportion deemed palatable. The daily intake of soil and feed was then estimated by weighing the remaining feed and estimating spilled feed weights. In addition to the possible variability in doses and estimates of dose resulting from this dosing procedure, there is also the possibility of occasional inhomogeneities in the soil/feed mixture, resulting in variable doses.

Soil Bioavailability Evaluations

TEQ Weighting

The two soil samples tested each contained a number of dioxin and/or furan contaminants, but for each soil, the total TEQ of the soil was dominated by two congeners (Table 2). For the Midland soil, the TEQ was dominated by TCDD and PeCDD, accounting together for approximately 75% of the total TEQ concentration. The TEQ concentration of the Tittabawassee soil was dominated by TCDF and 4-PeCDF, again together accounting for 75% of the TEQ.

Table 16 provides estimates of the overall relative bioavailability for the two soils compared to the corn oil reference material based on weighting the RBA estimates for individual congeners in proportion to their contribution to the total soil TEQ. RBA estimates based on the rat model and on the swine model under the two assumptions regarding non-detects are presented.

Absolute Bioavailability Estimates

This pilot study allows direct estimates of relative biovailability from soil compared to corn oil (rats and swine) or, for the Midland soil, compared to diet (rats only). The absolute bioavailability of the congeners may be of of interest for the risk assessment of these soils if soil exposure is compared to established intake targets for humans that rely on absolute estimates of dose or body burden (for example, the WHO/JECFA or ECSCF TDI values). The absolute bioavailability of the tested congeners from soil can be estimated if the absolute bioavailability

from the corn oil reference material is known. Rats and mice absorb between 60% and 90% of TCDD from oral administration in corn oil (Hurst et al. 2000; Diliberto et al. 1996, 2001). Other congeners with 4 to 6 chlorine atoms probably have similar absorption rates from corn oil, although congeners with 7 and 8 chlorine atoms may be much more poorly absorbed from corn oil (Birnbaum and Couture 1988).

Table 16 presents estimates of absolute bioavailability for the tested congeners and soils, assuming that the PCDD/Fs in the corn oil reference material have absolute bioavailability of 80%. The absolute bioavailability estimates of the soils would decrease if the absolute bioavailability of the cornoil–administered compounds is lower than 80%, and would increase if the absolute bioavailability of corn oil–administered compounds is greater than 80%.

Comparison with *In Vitro* Bioaccessibility Data

A sample of the Midland soil tested in rats and swine (CC-S-27) was evaluated previously for dioxin/furan bioaccessibility using an *in vitro* assay (Ruby et al. 2002). This assay measured the ability of a synthetic digestive fluid in an *in vitro* system to disassociate dioxin and furan congeners from soil. Such a test could serve as a predictor of the fraction of contaminant likely to be available for absorption in the gastrointestinal tract. Congener-specific bioaccessibility estimates ranged from about 16% to 26% of the total soil contamination for the Midland soil (Table 16). These estimates are similar to, but slightly lower than, the estimated absolute bioavailability of this soil based on the swine results. No Tittabawassee River flood plain soil was evaluated using the bioaccessibility assay, so no results are available for comparison to the flood plain soil test results presented here.

Conclusions and Recommendations for Final Study Design

The RBA estimates derived in this pilot study based on the rat model cannot be relied upon due to differential enzyme induction between soil and reference groups. To our knowledge, no previous evaluations of relative bioavailability for PCDD/Fs in soil in rats have measured EROD or MROD activity in the study animals. This suggests the possibility that previous bioavailability estimates may have been influenced by this factor as well.

The RBA estimates for the Midland soil based on the swine model also suffer from limitations due to the low tissue concentrations attained and failure to consistently exceed analytical detection limits. However, there are no *a priori* reasons to reject the swine-based RBA estimates for the Tittabawassee River flood plain soil compounds.

The data developed in this pilot study indicate that either of these animal models could potentially be used to assess PCDD/F bioavailability and provide a basis for developing a final study design that can be used to evaluate a selection of soils from both Midland and the Tittabawassee River flood plain.

Following are our recommendations for a final study design.

1. Choose a single animal model for future studies. Based on a variety of considerations, the rat model may be more practical for further studies. The rats are a more sensitive model based on attained tissue concentrations for a given soil concentration, and this will be important in future studies. The Midland soil tested, CC-S-27, is toward the upper end of TCDD and TEQ concentrations for Midland city soils analyzed to date. Even if a higher rate of soil dosing can be achieved with the swine, the swine model still might not be sensitive enough to obtain detectable tissue levels using Midland soils with lower TCDD or TEQ concentrations, which would greatly limit the Midland soil selection for future testing. Although achieving good control over the dosing rate of soil for the rats is more complicated than for the swine, this issue should be surmountable based on the experience gained during the pilot study. In addition, the results of this pilot study exhibited good reproducibility from one rat pair to the next, with relatively low CVs on the mean RBA estimates for all congeners. This indicates good inter-animal reproducibility with the current rat study design. In addition, rats have a long history of use as a dioxin bioavailability model, whereas swine, although widely used for assessing bioavailability of lead and arsenic, have almost no track record as a model for lipophilic compounds. Finally, although the RBA estimates derived in this pilot study are questionable due to the enzyme activity differences among groups, these preliminary data suggest that, for the congeners of greatest concern, the rats are producing greater RBA estimates than the swine. The rats would therefore be a conservative choice for future bioavailability studies.

   If rats are chosen as the model for use in further studies, several specific study design changes should be made:

- Reduce administered doses somewhat for soils with TEQ concentrations above 500 ppt TEQ, to reduce enzyme induction but still maintain detectable, quantifiable tissue levels. The administered dose of Tittabawassee River flood plain soil used in this study was more than sufficient to produce detectable, reproducible tissue concentrations of the compounds of interest. The Midland soil used here consistently produced quantifiable liver concentrations, and adipose tissue concentrations were consistently above detection limits but were sometimes below the analytical lower calibration limit.

- Match oil gavage reference doses to anticipated *absorbed* doses of soil congeners as closely as possible. This involves three adjustments to the current protocol:

    1. Match reference-dose material to mixed soil/feed analysis results, rather than trying to match both materials to the "target" dosing concentrations.

    2. In addition, when establishing target congener concentrations for the reference soil, reduce the expected soil/feed consumption rate to 18 g/day, consistent with what was observed in the pilot study for both soil/feed groups.

    3. Account for the range of likely relative bioavailability in choosing target gavage oil concentrations and doses. That is, if the relative bioavailability is expected to be in the range of 25% to 50% for soil compared to reference corn oil materials, the administered dose of compounds in the reference corn oil material should probably be reduced by 50% to 75% compared to the *administered* soil dose, to try to assure similar *absorbed* doses between the two groups. This approach should minimize any differences in enzyme induction between soil and reference groups.

- Omit the reference feed study group, because the results in this pilot study are consistent with conventional assumptions regarding bioavailability from feed, and two reference groups are unnecessary going forward.

However, if swine are chosen, the following protocol changes should be considered:

- Increase administered dose as much as possible to ensure tissue concentrations above detection limits.

- Consider doing an intravenous comparison group for one soil each from Midland and Tittabawassee to assess the absolute bioavailability of the corn oil-administered compounds.

2. Choose one tissue (either liver or fat) to reduce study costs in the future. The choice of tissue would depend on the choice of animal model.

In the swine model, in the dose ranges used in this pilot study, adipose tissue accumulated a much greater fraction of administered dose and exhibited a greater rate of detectable tissue levels (Figure 7).

However, in rats, the fraction of retained dose of the two predominant congeners, TCDD and PeCDD, was similar between liver and adipose tissue, while the higher chlorinated PCDDs and the 4-PeCDF were found predominantly in the liver (Figure 3). In addition, the RBA estimates derived based on liver tissue alone vs. adipose tissue alone were very consistent in the rat for both soils, so a single tissue could be chosen. The liver tissue is the simplest tissue to collect. In addition, livers can be weighed directly, so the total mass of the tissue compartment can be measured rather than estimated (as was done for the adipose tissue weight). Finally, if liver tissue is the basis for comparison, it will not be necessary to use pairs of rats rather than single animals for the tissue collection, because this was done to facilitate collection of sufficient fat tissue for analysis.

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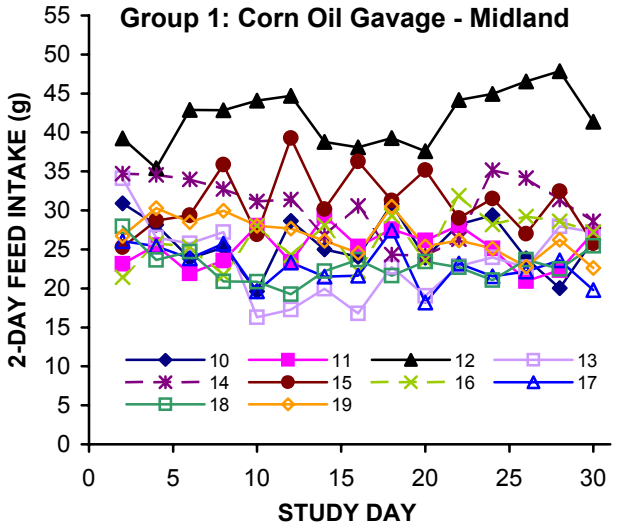
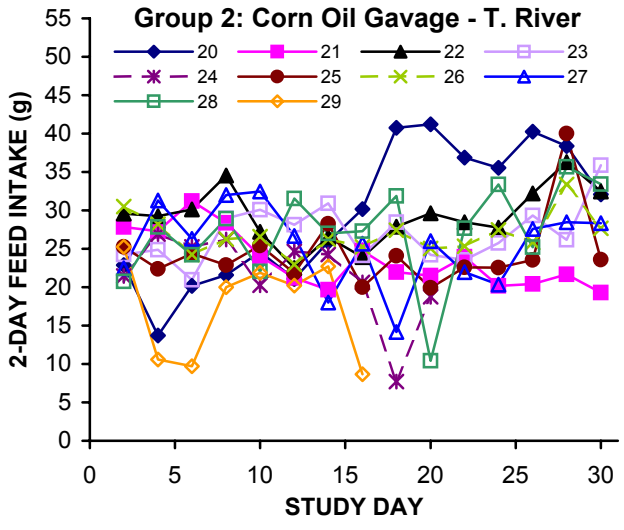
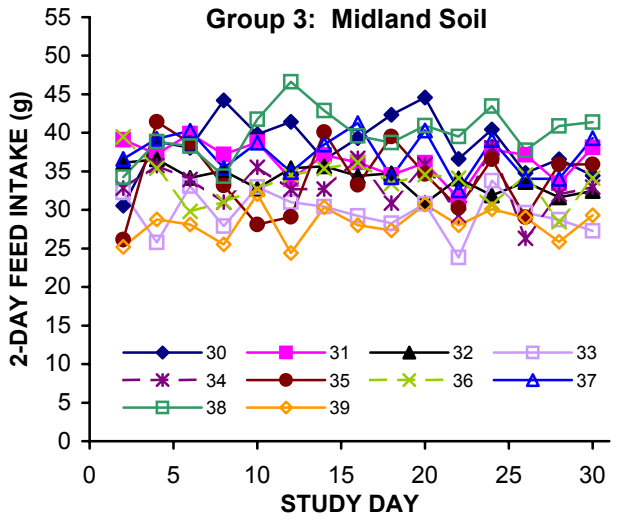
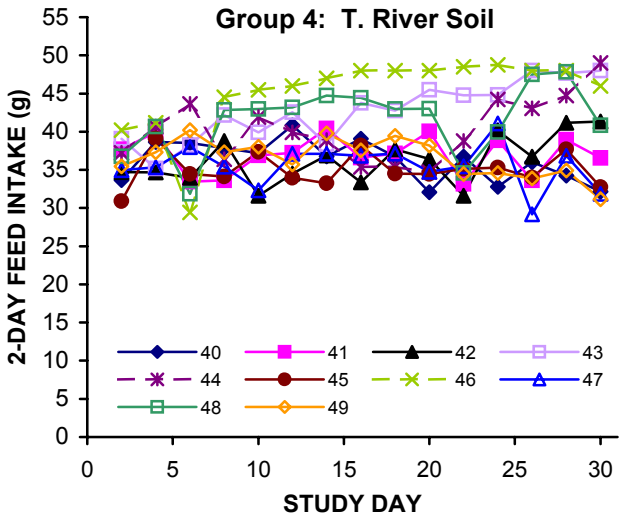
Figures









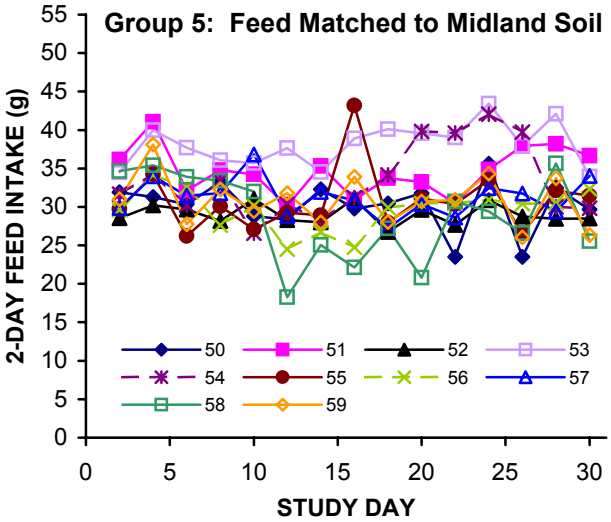


Figure 1. Feed intake for the rat pilot study

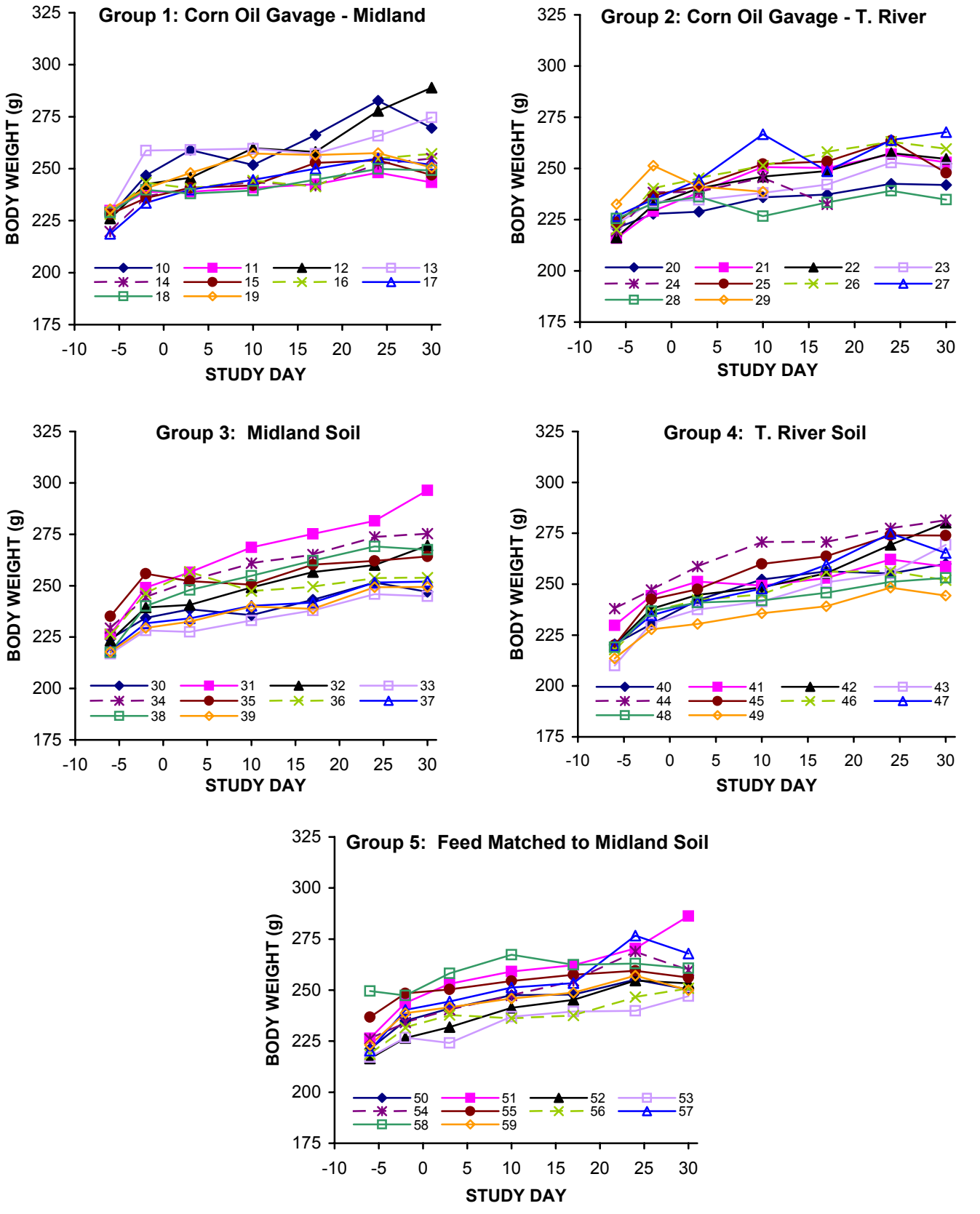


Figure 2. Body weights for the rat pilot study

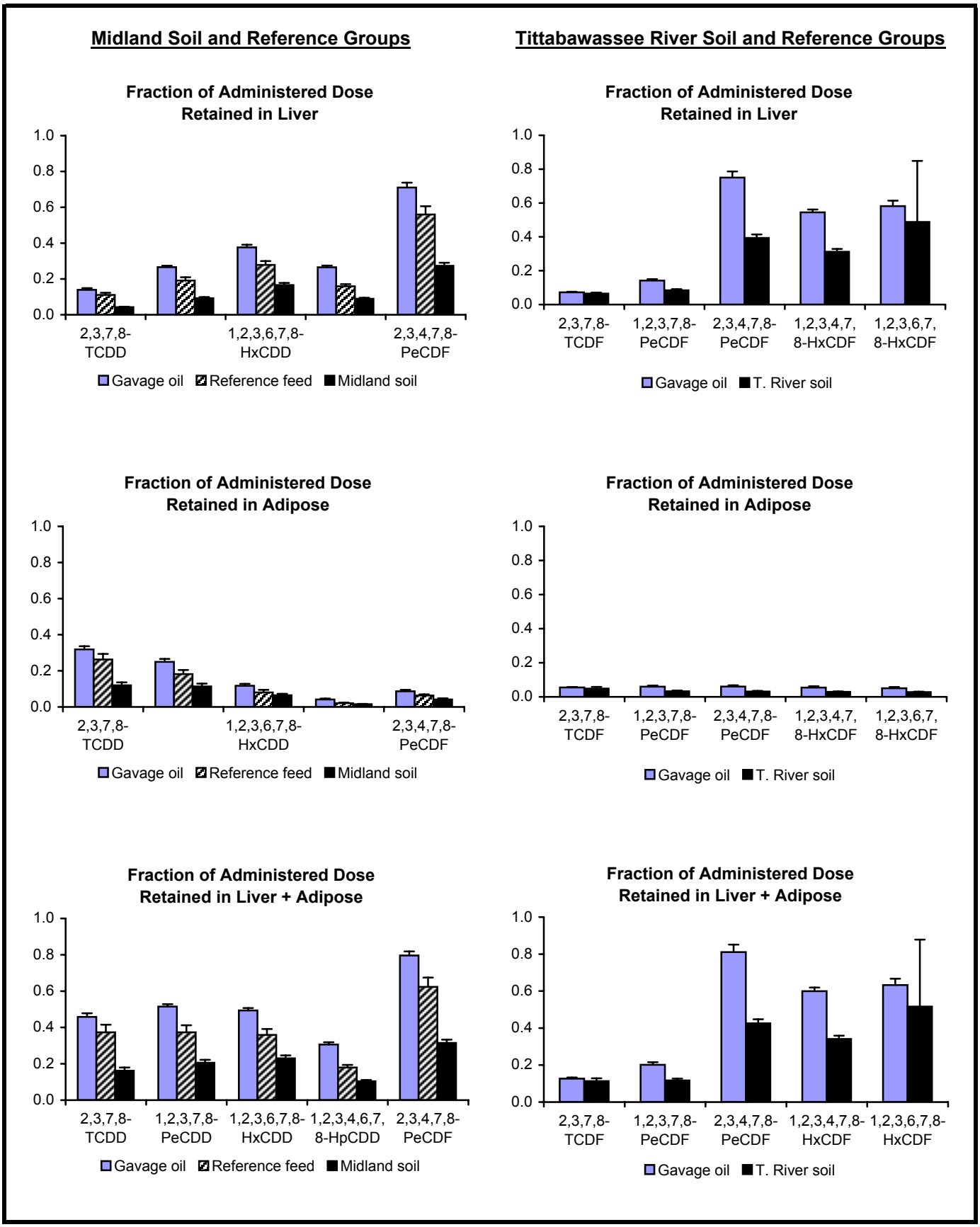
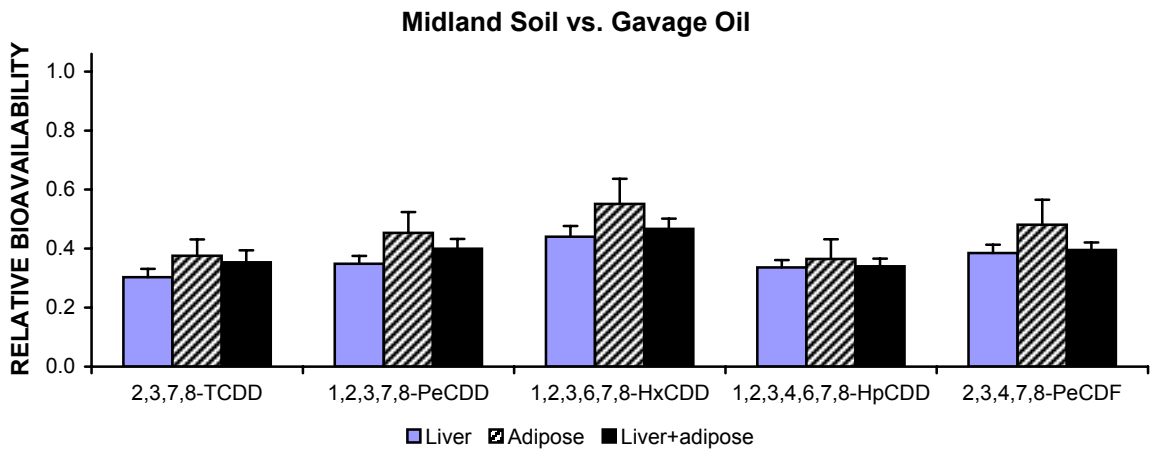
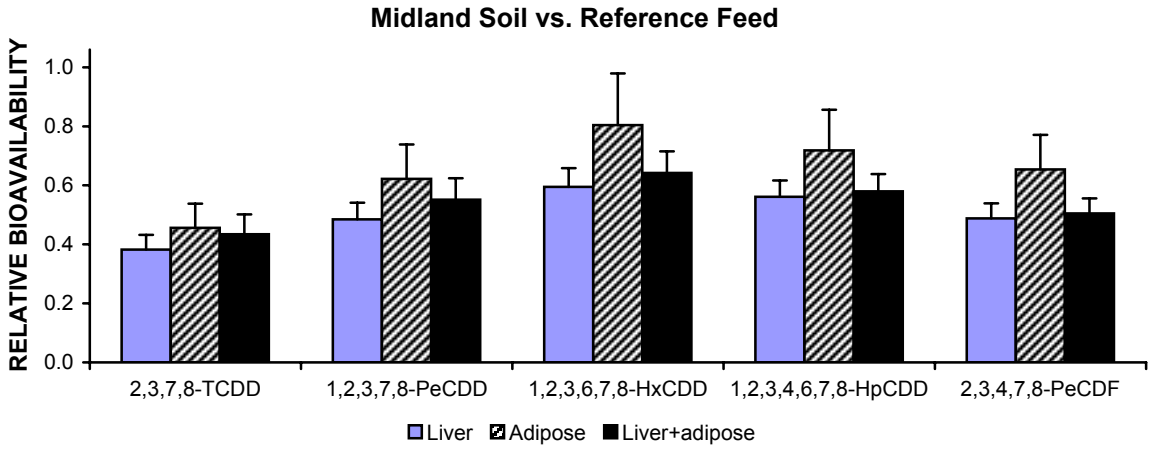
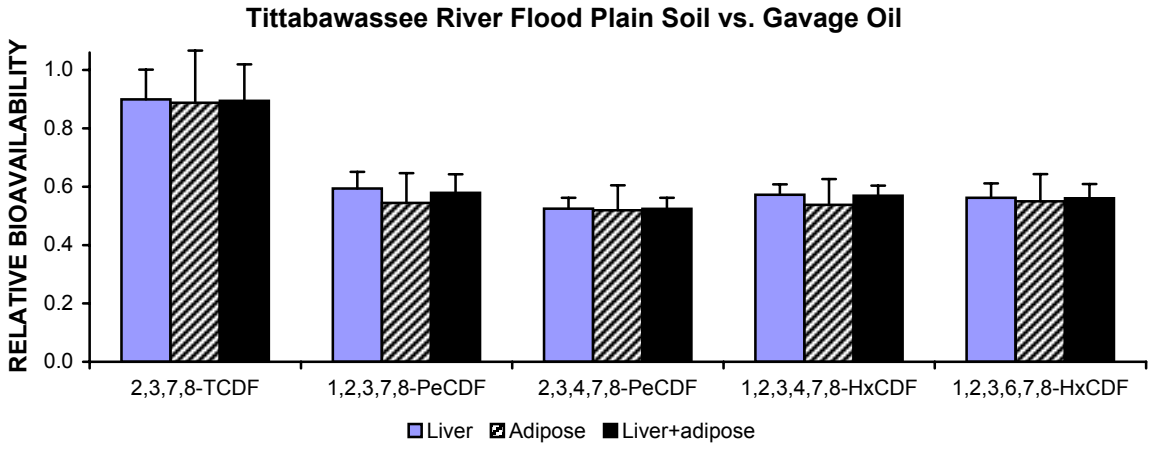


Figure 3. Distribution of administered doses in rat tissues







One outlier excluded for 1,2,3,6,7,8-HxCDF.

Figure 4. Relative bioavailability estimates for the rat pilot study

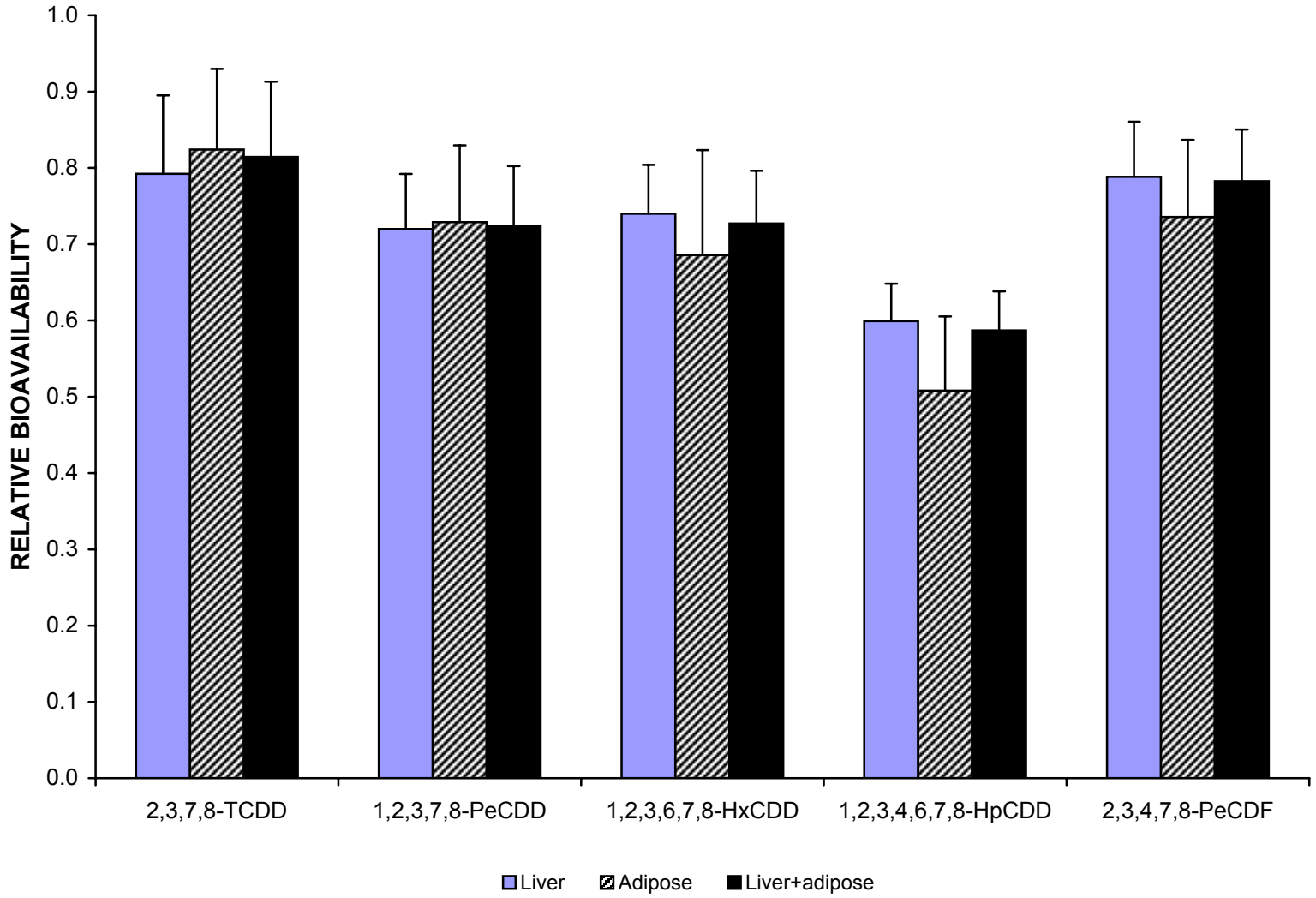


Figure 5. Relative bioavailability of the feed reference mixture compared to the corn oil reference mixture for the Midland soil

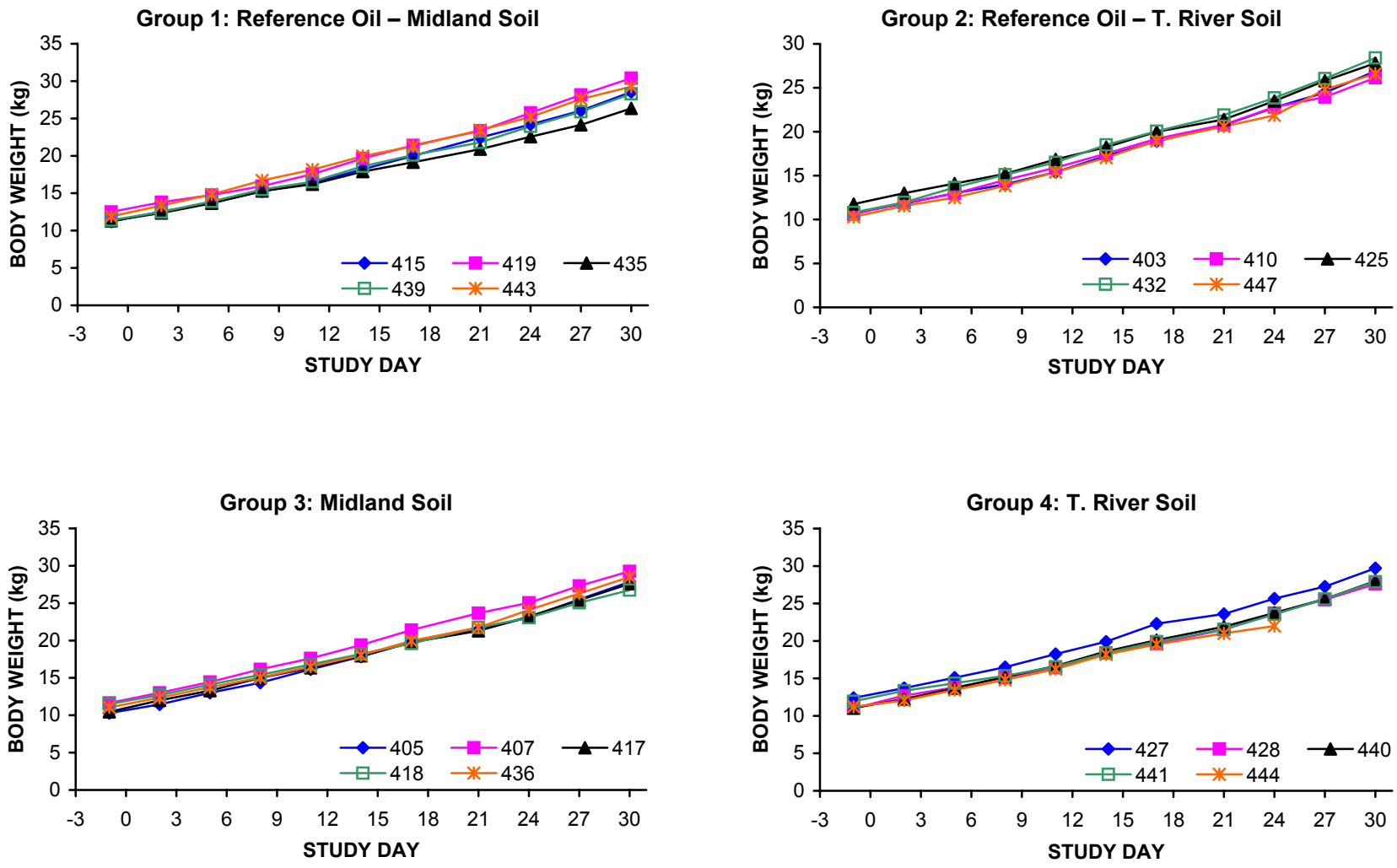


Figure 6. Body weights for the swine pilot study

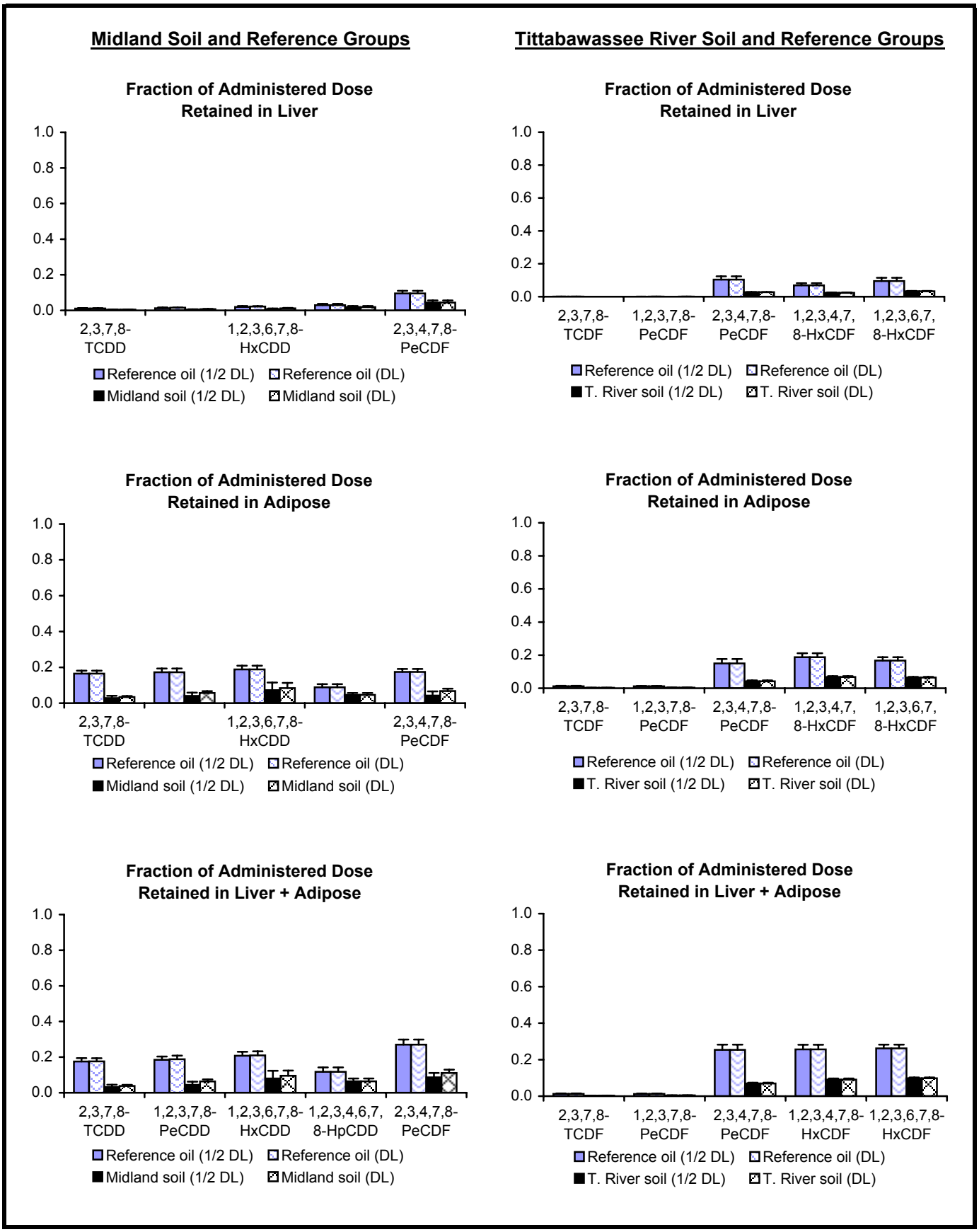
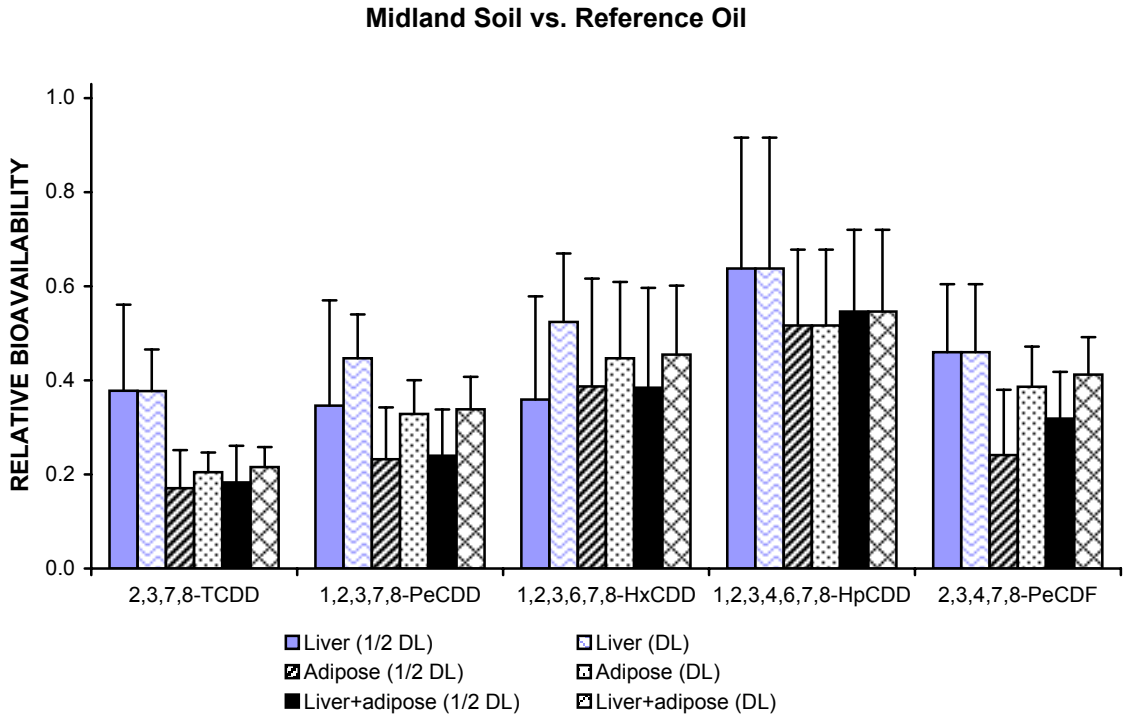
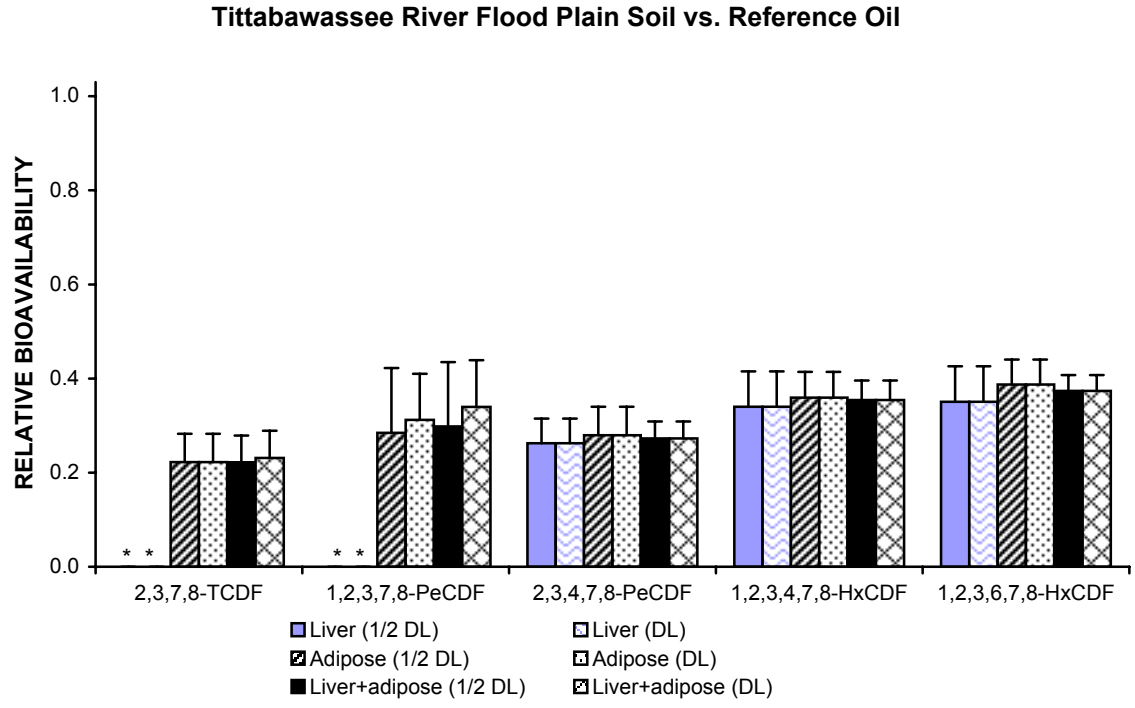


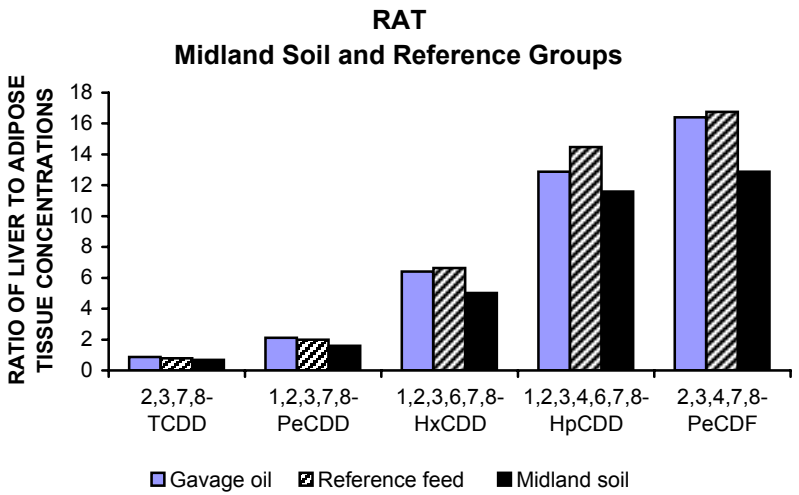
Figure 7. Distribution of administered doses in swine tissues

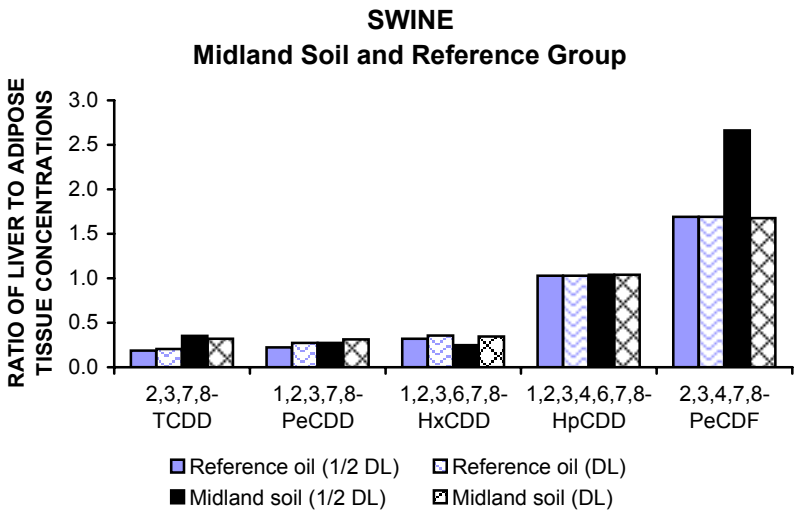


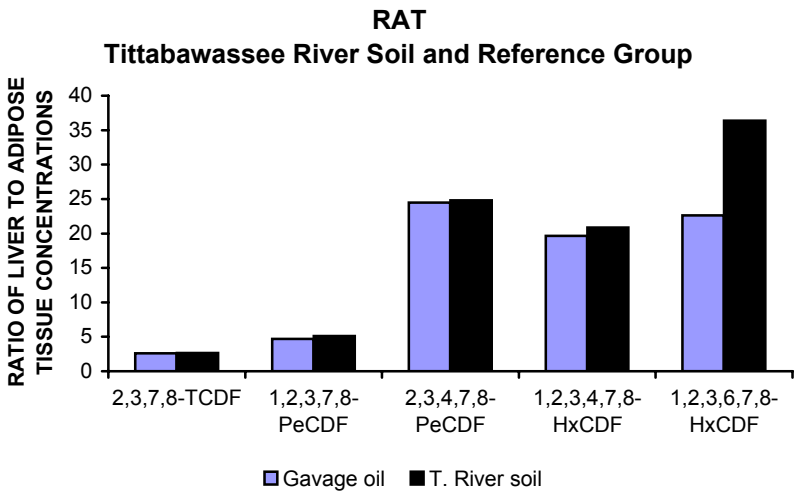


* Liver tissue concentrations were undetected in all soil group animals.

Figure 8. Relative bioavailability estimates for the swine pilot study







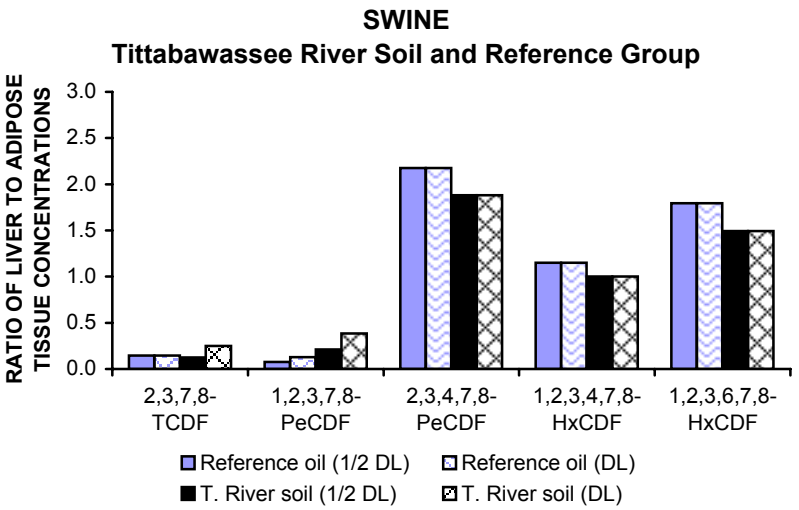
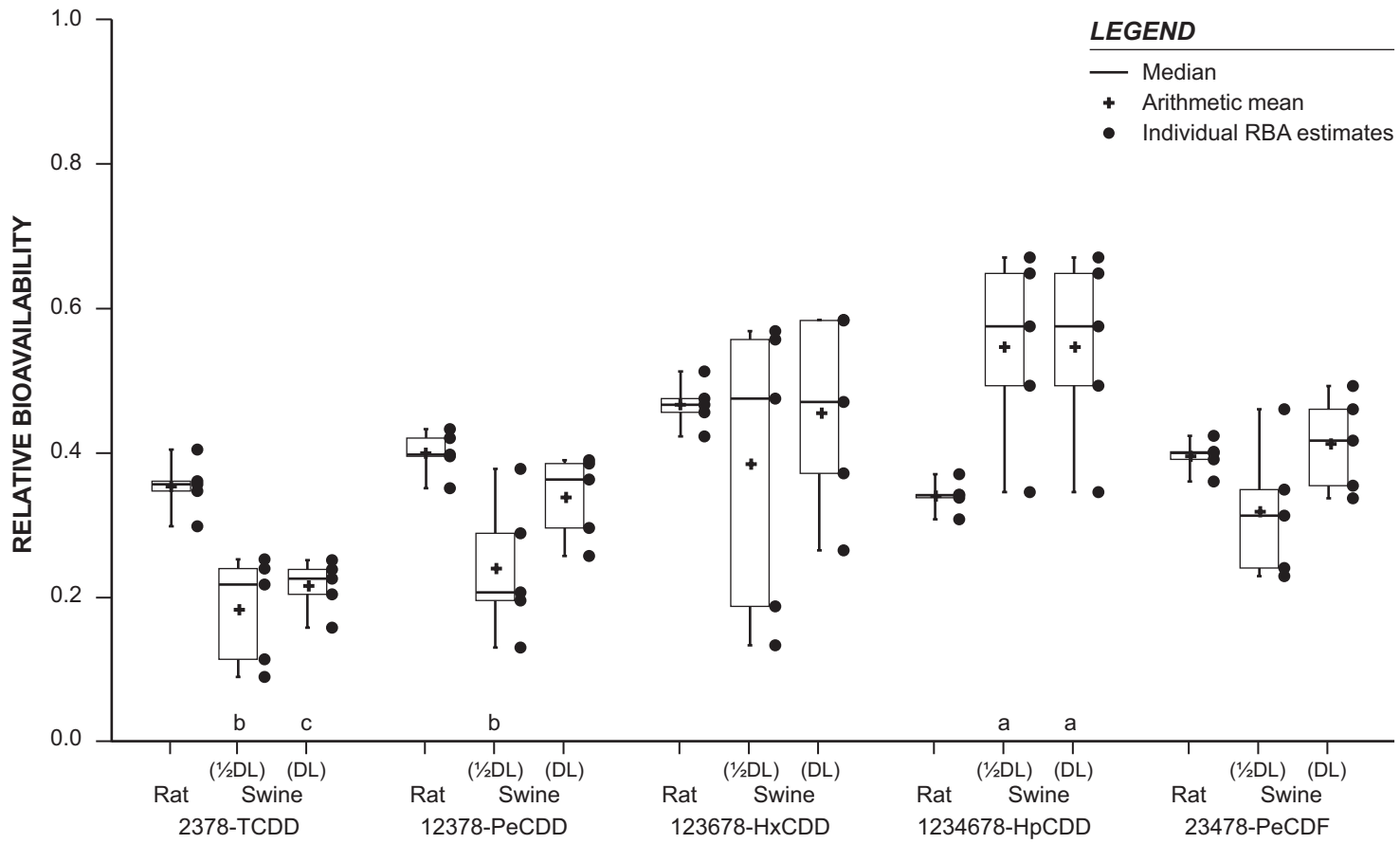


Figure 9. Ratio of liver to adipose tissue concentrations in the rat and swine pilot study

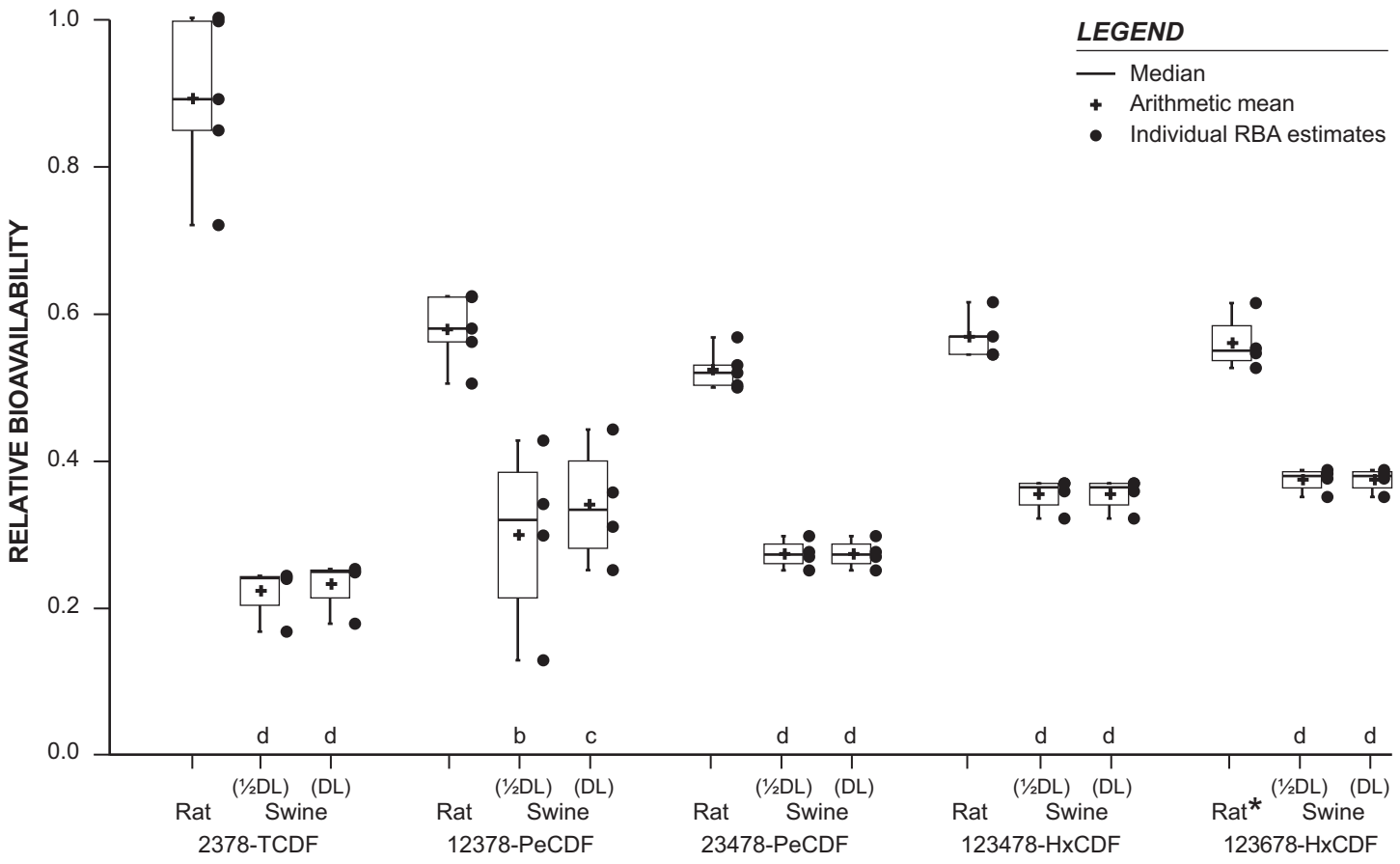


P-values:
Difference between rat and swine RBAs

a = p<0.05
b = p<0.01
c = p<0.001
d = p≤0.0001

Notes:
RBA estimates plotted for each animal (swine) or pair of animals (rats) based on the fraction of administered dose retained in liver plus adipose tissue compared to the average fraction of administered dose retained in liver plus adipose tissue in the respective corn oil reference group
½DL—Calculations performed using one-half the detection limit for non-detects
DL—Calculations performed using the detection limit for non-detects

Figure 10. Relative bioavailability estimates for the Midland soil in rats and swine



P-values:
Difference between rat and swine RBAs

a = p<0.05
b = p<0.01
c = p<0.001
d = p≤0.0001

Notes:

RBA estimates plotted for each animal (swine) or pair of animals (rats) based on the fraction of administered dose retained in liver plus adipose tissue compared to the average fraction of administered dose retained in liver plus adipose tissue in the respective corn oil reference group

½DL—Calculations performed using one-half the detection limit for non-detects

DL—Calculations performed using the detection limit for non-detects

*One outlier excluded from rat RBA estimate

Figure 11. Relative bioavailability estimates for the Tittabawassee River flood plain soil in rats and swine

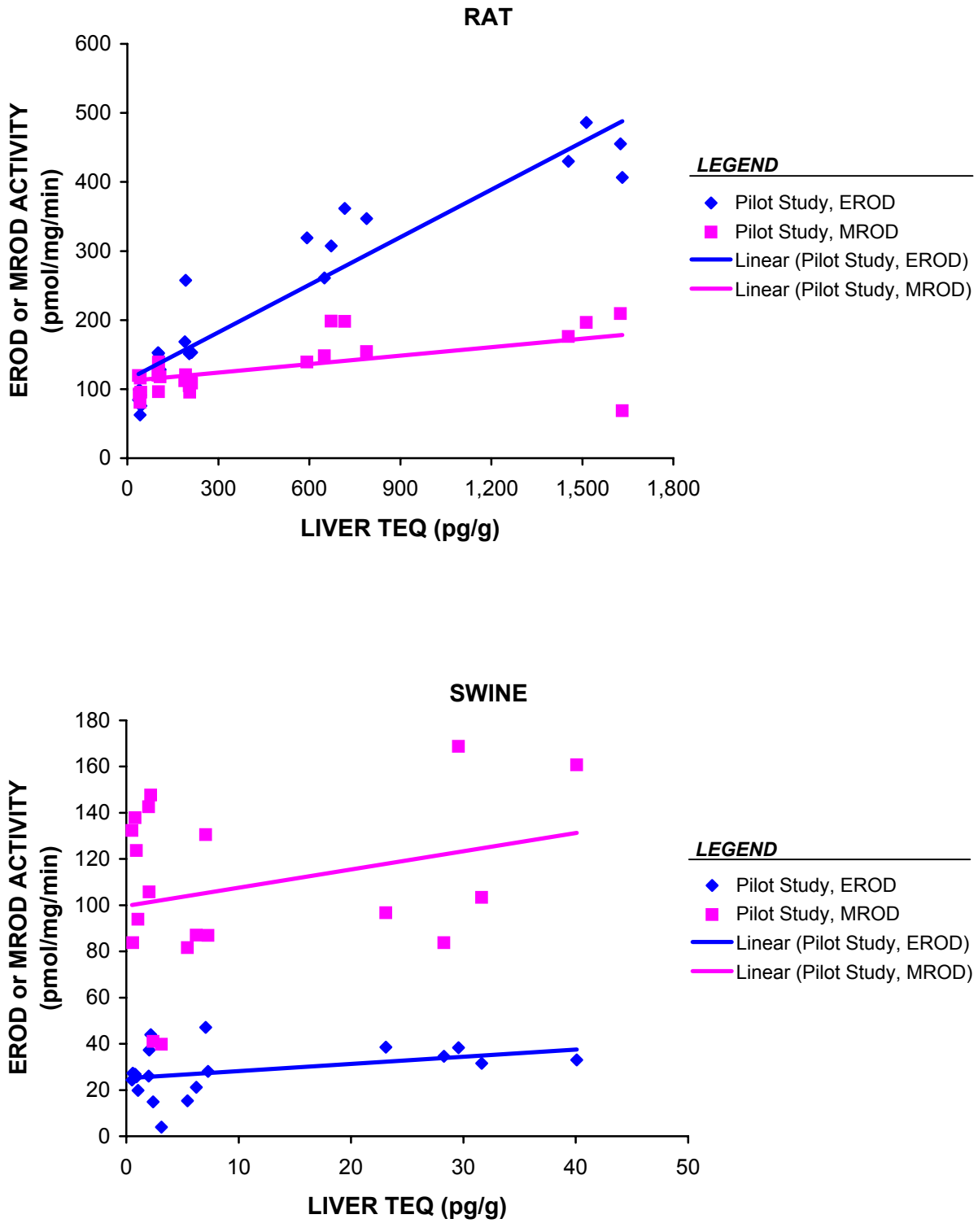


Figure 12. Enzyme activity in rat and swine liver microsomes for the pilot study

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DataEROD_MROD_Pig-rat_09-2004_samples.xls plot 2/24/2005 (3:10 PM)

Tables

Table 1. PCDD/F concentrations in candidate pilot study soils (<250 µm)

Sample Location:		Midland - 1		Midland - 2		N. of Caldwell Boat Launch		Imerman Park 1		Imerman Park 2	
Sample ID:		MNE02765		MNE02766		MIC02767		THT02768		THT02769	
Date:		6/25/2004		6/28/2004		6/28/2004		6/25/2004		6/28/2004	
Analyte	WHO TEF	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)
PCDDs/Fs											
2,3,7,8-TCDD	1	15.2	15.2	59.5	59.5	2.01	2.01	5.51	5.51	4.43	4.43
1,2,3,7,8-PeCDD	1	16.8	16.8	33.3	33.3	2.15 *J*	2.15	6.02	6.02	5.05	5.05
1,2,3,4,7,8-HxCDD	0.1	12.5	1.25	29.2	2.92	1.77 *J*	0.177	3.72 *J*	0.372	3.72 *J*	0.372
1,2,3,6,7,8-HxCDD	0.1	35.6	3.56	83.8	8.38	9.75	0.975	28.7	2.87	17.9	1.79
1,2,3,7,8,9-HxCDD	0.1	24.3	2.43	50.5	5.05	3.65 *J*	0.365	7.60	0.760	6.57	0.657
1,2,3,4,6,7,8-HpCDD	0.01	866	8.66	1,590	15.9	209	2.09	606	6.06	356	3.56
OCDD	0.0001	9,110 *E*	0.911	16,900 *E*	1.69	2,360	0.236	6,300	0.630	3,540	0.354
2,3,7,8-TCDF	0.1	4.94	0.494	69.5	6.95	64.3	6.43	2,160 *E*	216	2,380 *E*	238
1,2,3,7,8-PeCDF	0.05	4.08 *J*	0.204	51.6	2.58	34.1	1.71	1,020	51.0	1,230	61.5
2,3,4,7,8-PeCDF	0.5	9.82	4.91	81.3	40.7	35.8	17.9	898	449	984	492
1,2,3,4,7,8-HxCDF	0.1	18.4 *B*	1.84	114 *B*	11.4	59.7 *B*	5.97	685 *B*	68.5	822 *B*	82.2
1,2,3,6,7,8-HxCDF	0.1	14.5 *D*	1.45	48.1 *D*	4.81	13.6	1.36	145 *D*	14.5	187 *D*	18.7
2,3,4,6,7,8-HxCDF	0.1	13.6	1.36	55.3	5.53	7.67	0.767	86.7	8.67	107	10.7
1,2,3,7,8,9-HxCDF	0.1	5.34	0.534	21.2	2.12	9.50	0.950	130	13.0	156	15.6
1,2,3,4,6,7,8-HpCDF	0.01	416	4.16	949	9.49	286	2.86	881	8.81	681	6.81
1,2,3,4,7,8,9-HpCDF	0.01	16.1	0.161	47.0	0.470	23.8	0.238	74.5	0.745	71.4	0.714
OCDF	0.0001	1,020 *B*	0.102	1,700 *B*	0.170	712 *B*	0.0712	2,040 *B,D*	0.204	1,140 *B*	0.114
TEQ (pg/g)			**64.0**		**211**		**46.3**		**853**		**943**

Table 1. (cont.)

Sample Location:		W. Michigan Park		Dow Corporate Center	
Sample ID:		SHL02770		CC-S-27	
Date:		6/28/2004		5/17/2002	
Analyte	WHO TEF	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)
PCDDs/Fs					
2,3,7,8-TCDD	1	6.47	6.47	163	163
1,2,3,7,8-PeCDD	1	6.60	6.60	71.8	71.8
1,2,3,4,7,8-HxCDD	0.1	3.10 *J*	0.310	30.1	3.01
1,2,3,6,7,8-HxCDD	0.1	17.2	1.72	80.8	8.08
1,2,3,7,8,9-HxCDD	0.1	6.25	0.625	57.5	5.75
1,2,3,4,6,7,8-HpCDD	0.01	320	3.20	1,700	17
OCDD	0.0001	3,260	0.326	17,100 *B,E*	1.71
2,3,7,8-TCDF	0.1	1,330	133	28.3	2.83
1,2,3,7,8-PeCDF	0.05	642	32.1	22.5	1.125
2,3,4,7,8-PeCDF	0.5	565	283	31.7	15.85
1,2,3,4,7,8-HxCDF	0.1	440 *B*	44.0	56.9	5.69
1,2,3,6,7,8-HxCDF	0.1	95.7	9.57	26.1	2.61
2,3,4,6,7,8-HxCDF	0.1	56.4	5.64	30.5	3.05
1,2,3,7,8,9-HxCDF	0.1	88.3	8.83	13.1	1.31
1,2,3,4,6,7,8-HpCDF	0.01	633	6.33	784	7.84
1,2,3,4,7,8,9-HpCDF	0.01	47.8	0.478	30.5	0.305
OCDF	0.0001	1,110 *B*	0.111	1,290	0.129
TEQ (pg/g)			**542**		**311**

Note: *B* – This compound was also detected in the method blank.
D – The amount reported is the maximum possible concentration due to possible chlorinated diphenylether interference.
E – The amount detected is above the Upper Calibration Limit of the instrument.
J – The amount detected is below the Lower Calibration Limit of the instrument.
TEQ – Toxicity Equivalence Concentration
WHO TEF – World Health Organization Toxicity Equivalence Factor

Table 2. PCDD/F and PCB concentrations in triplicate samples of pilot study test soils (<250 µm)

Sample Location:		Dow Corporate Center						
Sample ID:		CC-S-27						
Date:		7/8/2004						
Tag Number:		57278	57279	57280	Mean	Coefficient of		
Analyte	WHO TEF	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	Variability (%)	TEQ (pg/g)	% of TEQ
PCDDs/Fs								
2,3,7,8-TCDD	1	139	125	130	131	5.4%	131	49%
1,2,3,7,8-PeCDD	1	65.4	67.6	67.6	66.9	1.9%	66.9	25%
1,2,3,4,7,8-HxCDD	0.1	31.3	28.4	27.4	29.0	7.0%	2.90	1.1%
1,2,3,6,7,8-HxCDD	0.1	78.2	71.6	70.7	73.5	5.6%	7.35	2.7%
1,2,3,7,8,9-HxCDD	0.1	50.2	50.0	48.6	49.6	1.8%	4.96	1.8%
1,2,3,4,6,7,8-HpCDD	0.01	1,220	1,110	1,170	1,167	4.7%	11.7	4.3%
OCDD	0.0001	14,700	13,000 *B,E*	13,900 *B,E*	13,867 *B,E*	6.1%	1.39	0.5%
2,3,7,8-TCDF	0.1	34.9	29.1 *D*	36.9	33.6	12%	3.36	1.3%
1,2,3,7,8-PeCDF	0.05	26.8	25.1	25.3	25.7	3.6%	1.29	0.5%
2,3,4,7,8-PeCDF	0.5	38.0	34.8	35.4	36.1	4.7%	18.0	6.7%
1,2,3,4,7,8-HxCDF	0.1	57.9	52.8	54.5	55.1	4.7%	5.51	2.0%
1,2,3,6,7,8-HxCDF	0.1	29.3 *D*	31.3 *D*	28.0 *D*	29.5 *D*	5.6%	2.95	1.1%
2,3,4,6,7,8-HxCDF	0.1	33.1	29.9	30.2	31.1	5.7%	3.11	1.2%
1,2,3,7,8,9-HxCDF	0.1	13.2	12.0	11.8	12.3	6.1%	1.23	0.5%
1,2,3,4,6,7,8-HpCDF	0.01	643	623	650 *D*	639	2.2%	6.39	2.4%
1,2,3,4,7,8,9-HpCDF	0.01	32.1	28.8	30.2	30.4	5.5%	0.304	0.1%
OCDF	0.0001	1,240	1,200	1,250	1,230	2.2%	0.123	0.05%
TEQ (pg/g)							**269**	
PCBs								
PCB-77	0.0001	145	--	--	145	--	0.0145	--
PCB-81	0.0001	20.7	--	--	20.7	--	0.00207	--
PCB-105	0.0001	590	--	--	590	--	0.059	--
PCB-114	0.0005	32.7	--	--	32.7	--	0.0164	--
PCB-106/118	0.0001	1,100	--	--	1,100	--	0.11	--
PCB-123	0.0001	32.1	--	--	32.1	--	0.00321	--
PCB-126	0.1	25.5	--	--	25.5	--	2.55	--
PCB-156	0.0005	151	--	--	151	--	0.0755	--
PCB-157	0.0005	47.6 [a]	--	--	47.6 [a]	--	0.0238	--
PCB-167	0.00001	63.4	--	--	63.4	--	0.000634	--
PCB-169	0.01	9.54 *U* [c]	--	--	9.54 *U* [c]	--	0.0954	--
PCB-189	0.0001	15.5	--	--	15.5	--	0.00155	--
TEQ (pg/g)							**2.95**	
Total TEQ (pg/g)							**272**	
Other Parameters								
Solids, Total (%)	--	--	--	--	99.2	--	--	--
pH (s.u.)	--	--	--	--	5.77	--	--	--
Carbon, Total Organic (%)	--	--	--	--	3.14	--	--	--
Grain Size (%)								
Coarse sand (250 µm – 2 mm)	--	--	--	--	31.1	--	--	--
Fine sand (106 – 250 µm)	--	--	--	--	44.9	--	--	--
Very fine sand (75 – 106 µm)	--	--	--	--	11.4	--	--	--
Percent silt (4 – 75 µm)	--	--	--	--	12.1	--	--	--
Percent clay (< 4 µm)	--	--	--	--	0.50	--	--	--

Table 2. (cont.)

Sample Location:		Imerman Park 2						
Sample ID:		THT02769						
Date:		7/8/2004						
Tag Number:		57273	57274	57275	Mean	Coefficient of		
Analyte	WHO TEF	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	Concentration (pg/g)	Variability (%)	TEQ (pg/g)	% of TEQ
PCDDs/Fs								
2,3,7,8-TCDD	1	4.70	4.90	4.77	4.79	2.1%	4.79	0.6%
1,2,3,7,8-PeCDD	1	5.36 *J*	4.87	5.16	5.13	4.8%	5.13	0.6%
1,2,3,4,7,8-HxCDD	0.1	4.30 *J*	2.92 *U*[b]	3.60 *J*	3.61 *J*	19%	0.361	0.04%
1,2,3,6,7,8-HxCDD	0.1	26.3	18.7	17.9	21.0	22%	2.10	0.2%
1,2,3,7,8,9-HxCDD	0.1	8.04 *J*	7.30	7.68	7.67	4.8%	0.767	0.09%
1,2,3,4,6,7,8-HpCDD	0.01	490	383	346	406	18%	4.06	0.5%
OCDD	0.0001	4,540	3,820 *B*	3,530 *B*	3,963 *B*	13%	0.396	0.05%
2,3,7,8-TCDF	0.1	2,550 *E*	1,950	1,950	2,150	16%	215	25%
1,2,3,7,8-PeCDF	0.05	1,320	965	943	1,076	20%	53.8	6.3%
2,3,4,7,8-PeCDF	0.5	1,060	808	780	883	17%	441	52%
1,2,3,4,7,8-HxCDF	0.1	869	654	635	719	18%	71.9	8.5%
1,2,3,6,7,8-HxCDF	0.1	196 *D*	151 *D*	144 *D*	164 *D*	17%	16.4	1.9%
2,3,4,6,7,8-HxCDF	0.1	112	88.0	85.9	95.3	15%	9.53	1.1%
1,2,3,7,8,9-HxCDF	0.1	171	121	119	137	22%	13.7	1.6%
1,2,3,4,6,7,8-HpCDF	0.01	842	670	657 *D*	723	14%	7.23	0.9%
1,2,3,4,7,8,9-HpCDF	0.01	83.6	60.5	60.8	68.3	19%	0.683	0.08%
OCDF	0.0001	1,530	1,160	1,100	1,263	18%	0.126	0.01%
TEQ (pg/g)							**847**	
PCBs								
PCB-77	0.0001	42.0	--	--	42.0	--	0.0042	--
PCB-81	0.0001	10.0	--	--	10.0	--	0.001	--
PCB-105	0.0001	145	--	--	145	--	0.0145	--
PCB-114	0.0005	67.0	--	--	67.0	--	0.0335	--
PCB-106/118	0.0001	354	--	--	354	--	0.0354	--
PCB-123	0.0001	17.8	--	--	17.8	--	0.00178	--
PCB-126	0.1	10.3	--	--	10.3	--	1.03	--
PCB-156	0.0005	54.8	--	--	54.8	--	0.0274	--
PCB-157	0.0005	12.7	--	--	12.7	--	0.00635	--
PCB-167	0.00001	25.4	--	--	25.4	--	0.000254	--
PCB-169	0.01	9.60 *U*[c]	--	--	9.60 *U*[c]	--	0.096	--
PCB-189	0.0001	12.5	--	--	12.5	--	0.00125	--
TEQ (pg/g)							**1.25**	
Total TEQ (pg/g)							**849**	
Other Parameters								
Solids, Total (%)	--	--	--	--	98.9	--	--	--
pH (s.u.)	--	--	--	--	7.69	--	--	--
Carbon, Total Organic (%)	--	--	--	--	2.73	--	--	--
Grain Size (%)								
Coarse sand (250 µm – 2 mm)	--	--	--	--	42.1	--	--	--
Fine sand (106 – 250 µm)	--	--	--	--	26.8	--	--	--
Very fine sand (75 – 106 µm)	--	--	--	--	8.78	--	--	--
Percent silt (4 – 75 µm)	--	--	--	--	21.4	--	--	--
Percent clay (< 4 µm)	--	--	--	--	0.86	--	--	--

(notes appear on following page)

Table 2. (cont.)

Note: *B* – This compound was also detected in the method blank.
D – The amount reported is the maximum possible concentration due to possible chlorinated diphenylether interference.
E – The amount detected is above the Upper Calibration Limit of the instrument.
J – The amount detected is below the Lower Calibration Limit of the instrument.
U – Not detected; value represents the sample-specific detection limit, unless noted otherwise.
TEQ – Toxicity Equivalence Concentration
WHO TEF – World Health Organization Toxicity Equivalence Factor
Highlighting indicates the five congeners in each sample that contribute most to the total TEQ
If more than half of the results for a chemical were qualified with a *B*, *D*, *E*, or *J*, then the associated mean concentration was also qualified.

[a] Taken from a dilution of the extract.
[b] Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).
[c] Nondetect reported to the reporting limit.

Table 3. PCDD/F concentrations in Rodent Lab Diet 5001 and corn oil

Sample ID:		Rodent Lab Diet 5001		Rodent Lab Diet 5001		Corn Oil (Spectrum Chemical)	
Date:		5/17/2004		8/25/2004		8/9/2004	
Analyte	WHO TEF	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)	Concentration (pg/g)	TEQ (pg/g)
PCDDs/Fs							
2,3,7,8-TCDD	1	0.143 *U*	0.143	0.152 *U*	0.152	0.0576 *U*	0.0576
1,2,3,7,8-PeCDD	1	0.268 *U*	0.268	0.532 *U*	0.532	0.0617 *U*	0.0617
1,2,3,4,7,8-HxCDD	0.1	0.278 *U*	0.0278	0.262 *U*	0.0262	0.206 *U*	0.0206
1,2,3,6,7,8-HxCDD	0.1	0.295 *U*	0.0295	0.283 *U*	0.0283	0.246 *U*	0.0246
1,2,3,7,8,9-HxCDD	0.1	0.275 *U*	0.0275	0.266 *U*	0.0266	0.190 *U*	0.0190
1,2,3,4,6,7,8-HpCDD	0.01	0.541 *J*	0.00541	0.934 *J*	0.00934	0.753	0.00753
OCDD	0.0001	8.97 *J*	0.000897	10.5	0.00105	7.12	0.000712
2,3,7,8-TCDF	0.1	0.279 *U*	0.0279	0.144 *U*	0.0144	0.0605 *U*	0.00605
1,2,3,7,8-PeCDF	0.05	0.195 *U*	0.00975	0.370 *U*	0.0185	0.187 *U*	0.00935
2,3,4,7,8-PeCDF	0.5	0.190 *U*	0.095	0.333 *U*	0.1665	0.161 *U*	0.0805
1,2,3,4,7,8-HxCDF	0.1	0.136 *U*[a]	0.0136	0.175 *U*	0.0175	0.126 *U*	0.0126
1,2,3,6,7,8-HxCDF	0.1	0.0920 *U*	0.0092	0.170 *U*	0.017	0.127 *U*	0.0127
2,3,4,6,7,8-HxCDF	0.1	0.110 *U*	0.011	0.190 *U*	0.019	0.112 *U*	0.0112
1,2,3,7,8,9-HxCDF	0.1	0.0651 *U*	0.00651	0.263 *U*	0.0263	0.118 *U*	0.0118
1,2,3,4,6,7,8-HpCDF	0.01	0.136 *U*	0.00136	0.177 *U*	0.00177	0.420 *U*	0.00420
1,2,3,4,7,8,9-HpCDF	0.01	0.0913 *U*	0.000913	0.268 *U*	0.00268	0.495 *U*	0.00495
OCDF	0.0001	0.429 *J*	4.29E-05	0.526 *U*	5.26E-05	0.218 *U*	2.18E-05
TEQ (pg/g)			**0.677**		**1.059**		**0.345**

Note: *J* – The amount detected is below the Lower Calibration Limit of the instrument.
U – Not detected; value represents the sample-specific detection limit, unless noted otherwise.
TEQ – Toxicity Equivalence Concentration
WHO TEF – World Health Organization Toxicity Equivalence Factor

[a] Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).

Table 4. PCDD/F and PCB concentrations in triplicate samples of blended rat diet

Sample ID:		Soil CC-S-27/Diet Blend (Test Article #1)					
Date:		8/25/2004					
		Pre-Dosing Analysis					
Analyte	WHO TEF	Bottom Concentration (pg/g)	Middle Concentration (pg/g)	Top Concentration (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	% of Expected Concentration
PCDDs/Fs							
2,3,7,8-TCDD	1	4.97	4.71	5.89	5.19	12%	79%
1,2,3,7,8-PeCDD	1	2.70	2.72 *J*	2.92	2.78	4.4%	83%
1,2,3,4,7,8-HxCDD	0.1	1.28 *J*	1.51 *J*	1.30 *U*[a]	1.36 *J*	9.3%	--
1,2,3,6,7,8-HxCDD	0.1	3.85	4.02	3.99	3.95	2.3%	107%
1,2,3,7,8,9-HxCDD	0.1	2.54 *J*	2.33 *J*	2.40 *J*	2.42 *J*	4.4%	--
1,2,3,4,6,7,8-HpCDD	0.01	74.6	75.6	78.3	76.2	2.5%	131%
OCDD	0.0001	921	973	929	941	3.0%	--
2,3,7,8-TCDF	0.1	1.27	1.15	1.71	1.38	21%	--
1,2,3,7,8-PeCDF	0.05	1.18 *J*	1.16 *J*	1.33 *J*	1.22 *J*	7.6%	--
2,3,4,7,8-PeCDF	0.5	1.59 *J*	1.67 *J*	1.52 *J*	1.59 *J*	4.7%	89%
1,2,3,4,7,8-HxCDF	0.1	2.63	2.53 *J*	2.58 *J*	2.58 *J*	1.9%	--
1,2,3,6,7,8-HxCDF	0.1	1.98 *J,D*	1.85 *J,D*	2.67 *D*	2.17 *J,D*	20%	--
2,3,4,6,7,8-HxCDF	0.1	1.33 *J*	1.28 *U*[a]	1.32 *J*	1.31 *J*	2.0%	--
1,2,3,7,8,9-HxCDF	0.1	0.633 *U*[a]	0.592 *J*	0.655 *J*	0.627 *J*	5.1%	--
1,2,3,4,6,7,8-HpCDF	0.01	30.1	28.2	29.9	29.4	3.6%	--
1,2,3,4,7,8,9-HpCDF	0.01	1.41 *J*	1.38 *J*	1.47 *J*	1.42 *J*	3.2%	--
OCDF	0.0001	64.9	62.2	65.8	64.3	2.9%	--
TEQ (pg/g)							
PCBs							
PCB-77	0.0001	--	7.62	--	7.62	--	--
PCB-81	0.0001	--	2.75 *U*[b]	--	2.75 *U*[b]	--	--
PCB-105	0.0001	--	49.5	--	49.5	--	--
PCB-114	0.0005	--	2.75 *U*[b]	--	2.75 *U*[b]	--	--
PCB-106/118	0.0001	--	129	--	129	--	--
PCB-123	0.0001	--	2.94	--	2.94	--	--
PCB-126	0.1	--	2.75 *U*[b]	--	2.75 *U*[b]	--	--
PCB-156	0.0005	--	16.3	--	16.3	--	--
PCB-157	0.0005	--	4.48	--	4.48	--	--
PCB-167	0.00001	--	7.68	--	7.68	--	--
PCB-169	0.01	--	2.75 *U*[b]	--	2.75 *U*[b]	--	--
PCB-189	0.0001	--	2.75 *U*[b]	--	2.75 *U*[b]	--	--
TEQ (pg/g)							
Total TEQ (pg/g)							

Table 4. (cont.)

Sample ID:	Soil CC-S-27/Diet Blend (Test Article #1)							
Date:	8/25/2004							
	Post-Dosing Analysis			Pre- and Post-Dosing Analysis				
Analyte	Concentration Rep 1 (pg/g)	Concentration Rep 2 (pg/g)	Concentration Rep 3 (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	TEQ (pg/g)	% of TEQ	% of Expected Concentration
PCDDs/Fs								
2,3,7,8-TCDD	3.57	3.78	3.46	4.40	22%	4.40	43%	67%
1,2,3,7,8-PeCDD	1.98 *J*	2.36 *J*	2.30 *J*	2.50 *J*	14%	2.50	24%	75%
1,2,3,4,7,8-HxCDD	1.19 *J*	1.85 *J*	1.24 *J*	1.40 *J*	18%	0.140	1.4%	--
1,2,3,6,7,8-HxCDD	2.83 *J*	3.88 *J*	2.94 *J*	3.59	15%	0.359	3.5%	97%
1,2,3,7,8,9-HxCDD	1.94 *J*	3.42 *J*	1.91 *J*	2.42 *J*	23%	0.242	2.4%	--
1,2,3,4,6,7,8-HpCDD	57.5	78.1	57.1	70.2	14%	0.702	6.9%	120%
OCDD	774	893	783	879	9.3%	0.088	0.9%	--
2,3,7,8-TCDF	0.960 *J*	1.10	0.904 *J*	1.18	25%	0.118	1.2%	--
1,2,3,7,8-PeCDF	0.832 *U*[a]	1.03 *J*	0.839 *J*	1.06 *J*	19%	0.0530	0.5%	--
2,3,4,7,8-PeCDF	1.34 *J*	1.35 *J*	1.25 *J*	1.45 *J*	11%	0.725	7.1%	81%
1,2,3,4,7,8-HxCDF	2.30 *J*	2.09 *J*	2.28 *J*	2.40 *J*	8.8%	0.240	2.3%	--
1,2,3,6,7,8-HxCDF	1.22 *J*	1.07 *J*	1.13 *J*	1.65 *J*	38%	0.165	1.6%	--
2,3,4,6,7,8-HxCDF	1.08 *J*	1.06 *J*	1.21 *J*	1.21 *J*	9.8%	0.121	1.2%	--
1,2,3,7,8,9-HxCDF	0.607 *J*	0.535 *J*	0.571 *U*	0.599 *J*	7.2%	0.0599	0.6%	--
1,2,3,4,6,7,8-HpCDF	28.2	29.8	27.5	29.0	3.8%	0.290	2.8%	--
1,2,3,4,7,8,9-HpCDF	1.31 *J*	1.69 *J*	1.53 *J*	1.47 *J*	9.1%	0.0147	0.1%	--
OCDF	60.3	62.7	59.0	62.5	4.2%	0.00625	0.1%	--
TEQ (pg/g)						**10.2**		
PCBs								
PCB-77	--	--	--	7.62	--	0.00076	--	--
PCB-81	--	--	--	2.75 *U*[b]	--	0.00028	--	--
PCB-105	--	--	--	49.5	--	0.00495	--	--
PCB-114	--	--	--	2.75 *U*[b]	--	0.00138	--	--
PCB-106/118	--	--	--	129	--	0.0129	--	--
PCB-123	--	--	--	2.94	--	0.00029	--	--
PCB-126	--	--	--	2.75 *U*[b]	--	0.275	--	--
PCB-156	--	--	--	16.3	--	0.00815	--	--
PCB-157	--	--	--	4.48	--	0.00224	--	--
PCB-167	--	--	--	7.68	--	7.7E-05	--	--
PCB-169	--	--	--	2.75 *U*[b]	--	0.0275	--	--
PCB-189	--	--	--	2.75 *U*[b]	--	0.00028	--	--
TEQ (pg/g)						**0.33**		
Total TEQ (pg/g)						**10.56**		

Table 4. (cont.)

Sample ID: / Date:		Soil THT02769/Diet Blend (Test Article #2) 8/4/2004					
		Pre-Dosing Analysis					
Analyte	WHO TEF	Bottom Concentration (pg/g)	Middle Concentration (pg/g)	Top Concentration (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	% of Expected Concentration
PCDDs/Fs							
2,3,7,8-TCDD	1	0.308 *J*	0.217 *U*[a]	0.258 *U*[a]	0.261 *U*[a]	17%	--
1,2,3,7,8-PeCDD	1	0.280 *J*	0.282 *U*[a]	0.240 *U*[a]	0.267 *U*[a]	8.9%	--
1,2,3,4,7,8-HxCDD	0.1	0.307 *U*	0.214 *J*	0.226 *J*	0.249 *J*	20%	--
1,2,3,6,7,8-HxCDD	0.1	1.33 *J*	1.21 *J*	1.34 *J*	1.29 *J*	5.6%	--
1,2,3,7,8,9-HxCDD	0.1	0.493 *J*	0.440 *J*	0.474 *J*	0.469 *J*	5.7%	--
1,2,3,4,6,7,8-HpCDD	0.01	24.7	23.3	26.0	24.7	5.5%	--
OCDD	0.0001	245	223 *B*	255 *B*	241 *B*	6.8%	--
2,3,7,8-TCDF	0.1	77.2	79.5	88.4	81.7	7.2%	76%
1,2,3,7,8-PeCDF	0.05	50.6	47.8	52.3	50.2	4.5%	93%
2,3,4,7,8-PeCDF	0.5	43.7	41.2	45.5	43.5	5.0%	98%
1,2,3,4,7,8-HxCDF	0.1	35.4	32.1 *B*	34.5 *B*	34.0 *B*	5.0%	95%
1,2,3,6,7,8-HxCDF	0.1	9.48	7.33 *B,D*	7.79 *B*	8.20 *B*	14%	100%
2,3,4,6,7,8-HxCDF	0.1	4.70	4.23	4.56	4.50	5.4%	--
1,2,3,7,8,9-HxCDF	0.1	6.79	6.07	6.47	6.44	5.6%	--
1,2,3,4,6,7,8-HpCDF	0.01	37.8	32.8 *B*	35.7 *B*	35.4 *B*	7.1%	--
1,2,3,4,7,8,9-HpCDF	0.01	3.52	2.99	3.36	3.29	8.3%	--
OCDF	0.0001	70.4	60.8	68.4	66.5	7.6%	--
TEQ (pg/g)							
PCBs							
PCB-77	0.0001	--	5.04	--	5.04	--	--
PCB-81	0.0001	--	2.71 *U*[b]	--	2.71 *U*[b]	--	--
PCB-105	0.0001	--	33.8	--	33.8	--	--
PCB-114	0.0005	--	3.47	--	3.47	--	--
PCB-106/118	0.0001	--	101	--	101	--	--
PCB-123	0.0001	--	2.71 *U*[b]	--	2.71 *U*[b]	--	--
PCB-126	0.1	--	2.71 *U*[b]	--	2.71 *U*[b]	--	--
PCB-156	0.0005	--	12.2	--	12.2	--	--
PCB-157	0.0005	--	3.32	--	3.32	--	--
PCB-167	0.00001	--	6.41	--	6.41	--	--
PCB-169	0.01	--	2.71 *U*[b]	--	2.71 *U*[b]	--	--
PCB-189	0.0001	--	2.71 *U*[b]	--	2.71 *U*[b]	--	--
TEQ (pg/g)							
Total TEQ (pg/g)							

Table 4. (cont.)

Sample ID:	Soil THT02769/Diet Blend (Test Article #2)							
Date:	8/4/2004							
	Post-Dosing Analysis			Pre- and Post-Dosing Analysis				
Analyte	Concentration Rep 1 (pg/g)	Concentration Rep 2 (pg/g)	Concentration Rep 3 (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	TEQ (pg/g)	% of TEQ	% of Expected Concentration
PCDDs/Fs								
2,3,7,8-TCDD	0.330 *J*	0.532 *U*	0.284 *U*[a]	0.322 *U*	34%	0.322	0.8%	--
1,2,3,7,8-PeCDD	0.264 *U*[a]	0.293 *U*[a]	0.371 *J*	0.288 *U*	15%	0.288	0.7%	--
1,2,3,4,7,8-HxCDD	0.482 *U*	0.510 *U*	0.442 *U*	0.364 *U*	36%	0.0364	0.1%	--
1,2,3,6,7,8-HxCDD	0.991 *J*	1.09 *J*	0.954 *J*	1.15 *J*	14%	0.115	0.3%	--
1,2,3,7,8,9-HxCDD	0.631 *U*	0.468 *J*	0.836 *U*	0.557 *J*	27%	0.0557	0.1%	--
1,2,3,4,6,7,8-HpCDD	22.5	22.6	23.2	23.7	5.8%	0.237	0.6%	--
OCDD	235	230	231	237	4.9%	0.0237	0.1%	--
2,3,7,8-TCDF	83.9	87.2	86.1	83.7	5.3%	8.37	21%	78%
1,2,3,7,8-PeCDF	51.7	52.0	51.4	51.0	3.3%	2.55	6.4%	95%
2,3,4,7,8-PeCDF	44.1	44.6	44.4	43.9	3.3%	22.0	55%	99%
1,2,3,4,7,8-HxCDF	33.8	35.2	34.0	34.2	3.5%	3.42	8.6%	95%
1,2,3,6,7,8-HxCDF	8.29	8.73	9.08	8.45	9.5%	0.845	2.1%	103%
2,3,4,6,7,8-HxCDF	4.65 *J*	4.82 *J*	4.86 *J*	4.64	4.9%	0.464	1.2%	--
1,2,3,7,8,9-HxCDF	6.45	7.43	6.78	6.67	6.9%	0.667	1.7%	--
1,2,3,4,6,7,8-HpCDF	34.7	35.9	35.7	35.4	4.6%	0.354	0.9%	--
1,2,3,4,7,8,9-HpCDF	3.41 *J*	3.62 *J*	3.76 *J*	3.44	7.7%	0.0344	0.1%	--
OCDF	73.5	74.6	73.0	70.1	7.3%	0.00701	0.02%	--
TEQ (pg/g)						**39.7**		
PCBs								
PCB-77	--	--	--	5.04	--	0.000504	--	--
PCB-81	--	--	--	2.71 *U*[b]	--	0.000271	--	--
PCB-105	--	--	--	33.8	--	0.00338	--	--
PCB-114	--	--	--	3.47	--	0.00174	--	--
PCB-106/118	--	--	--	101	--	0.0101	--	--
PCB-123	--	--	--	2.71 *U*[b]	--	0.000271	--	--
PCB-126	--	--	--	2.71 *U*[b]	--	0.271	--	--
PCB-156	--	--	--	12.2	--	0.00610	--	--
PCB-157	--	--	--	3.32	--	0.00166	--	--
PCB-167	--	--	--	6.41	--	6.41E-05	--	--
PCB-169	--	--	--	2.71 *U*[b]	--	0.0271	--	--
PCB-189	--	--	--	2.71 *U*[b]	--	0.000271	--	--
TEQ (pg/g)						**0.32**		
Total TEQ (pg/g)						**40.1**		

Table 4. (cont.)

Sample ID: / Date:		Acetone Reference Mixture/Feed Blend (Test Article #3) 8/4/2004					
		Pre-Dosing Analysis					
Analyte	WHO TEF	Bottom Concentration (pg/g)	Middle Concentration (pg/g)	Top Concentration (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	% of Expected Concentration
PCDDs/Fs							
2,3,7,8-TCDD	1	5.56	5.30	5.44	5.43	2.4%	83%
1,2,3,7,8-PeCDD	1	3.29	3.38	3.47	3.38	2.7%	101%
1,2,3,4,7,8-HxCDD	0.1	0.0566 *U*	0.0629 *U*	0.0962 *U*	0.0719 *U*	30%	--
1,2,3,6,7,8-HxCDD	0.1	4.37	4.23	4.49	4.36	3.0%	118%
1,2,3,7,8,9-HxCDD	0.1	0.222 *J*	0.218 *J*	0.219 *J*	0.220 *J*	0.9%	--
1,2,3,4,6,7,8-HpCDD	0.01	55.1	54.9	55.9	55.3	1.0%	95%
OCDD	0.0001	8.66 *B*	8.54 *B*	8.99 *B*	8.73 *B*	2.7%	--
2,3,7,8-TCDF	0.1	0.0834 *J*	0.0934 *J*	0.0910 *J*	0.0893 *J*	5.8%	--
1,2,3,7,8-PeCDF	0.05	0.0533 *U*	0.0454 *U*	0.0414 *U*	0.0467 *U*	13%	--
2,3,4,7,8-PeCDF	0.5	1.87 *J*	1.82 *J*	1.87 *J*	1.85 *J*	1.6%	104%
1,2,3,4,7,8-HxCDF	0.1	0.0235 *U*	0.0244 *U*	0.0298 *U*	0.0259 *U*	13%	--
1,2,3,6,7,8-HxCDF	0.1	0.0251 *U*	0.0233 *U*	0.0297 *U*	0.0260 *U*	13%	--
2,3,4,6,7,8-HxCDF	0.1	0.0277 *U*	0.0265 *U*	0.0331 *U*	0.0291 *U*	12%	--
1,2,3,7,8,9-HxCDF	0.1	0.0363 *U*	0.0381 *U*	0.0435 *U*	0.0393 *U*	9.5%	--
1,2,3,4,6,7,8-HpCDF	0.01	0.115 *J,B*	0.0805 *J,B*	0.156 *U*	0.117 *J*	32%	--
1,2,3,4,7,8,9-HpCDF	0.01	0.0776 *U*	0.0469 *U*	0.168 *U*	0.0975 *U*	65%	--
OCDF	0.0001	0.167 *J*	0.156 *U*[a]	0.168 *J*	0.164 *J*	4.1%	--
TEQ (pg/g)							
PCBs							
PCB-77	0.0001	--	3.44	--	3.44	--	--
PCB-81	0.0001	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
PCB-105	0.0001	--	31.1	--	31.1	--	--
PCB-114	0.0005	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
PCB-106/118	0.0001	--	91.6	--	91.6	--	--
PCB-123	0.0001	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
PCB-126	0.1	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
PCB-156	0.0005	--	10.8	--	10.8	--	--
PCB-157	0.0005	--	3.07	--	3.07	--	--
PCB-167	0.00001	--	5.50	--	5.50	--	--
PCB-169	0.01	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
PCB-189	0.0001	--	2.90 *U*[b]	--	2.90 *U*[b]	--	--
TEQ (pg/g)							
Total TEQ (pg/g)							

Table 4. (cont.)

Sample ID:	Acetone Reference Mixture/Feed Blend (Test Article #3)							
Date:	8/4/2004							
	Post-Dosing Analysis			Pre- and Post-Dosing Analysis				
Analyte	Concentration Rep 1 (pg/g)	Concentration Rep 2 (pg/g)	Concentration Rep 3 (pg/g)	Mean Concentration (pg/g)	Coefficient of Variability (%)	TEQ (pg/g)	% of TEQ	% of Expected Concentration
PCDDs/Fs								
2,3,7,8-TCDD	5.64	5.67	5.63	5.54	2.6%	5.54	50%	84%
1,2,3,7,8-PeCDD	3.57 *J*	3.47 *J*	3.83 *J*	3.50	5.3%	3.50	31%	105%
1,2,3,4,7,8-HxCDD	0.646 *U*	0.580 *U*	0.272 *U*	0.286 *U*	93.2%	0.0286	0.3%	--
1,2,3,6,7,8-HxCDD	4.65 *J*	4.58 *J*	4.63 *J*	4.49	3.7%	0.449	4.0%	121%
1,2,3,7,8,9-HxCDD	0.688 *U*	0.474 *U*	0.691 *U*	0.419	55.4%	0.0419	0.4%	--
1,2,3,4,6,7,8-HpCDD	56.7	55.1	55.7	55.6	1.2%	0.556	5.0%	95%
OCDD	8.22 *J*	8.76 *J*	9.07 *J*	8.71	3.6%	0.000871	0.008%	--
2,3,7,8-TCDF	0.365 *U*	0.440 *U*	0.155 *J*	0.205 *J*	76.8%	0.0205	0.2%	--
1,2,3,7,8-PeCDF	0.380 *U*	0.445 *U*	0.509 *U*	0.246 *U*	90.3%	0.0123	0.1%	--
2,3,4,7,8-PeCDF	1.81 *J*	2.11 *J*	1.98 *J*	1.91 *J*	6.0%	0.955	8.6%	107%
1,2,3,4,7,8-HxCDF	0.139 *U*	0.129 *U*	0.0958 *U*	0.0736 *U*	73.7%	0.00736	0.1%	--
1,2,3,6,7,8-HxCDF	0.0898 *U*	0.129 *U*	0.0961 *U*	0.0655 *U*	69.1%	0.00655	0.1%	--
2,3,4,6,7,8-HxCDF	0.121 *U*	0.137 *U*	0.105 *U*	0.0751 *U*	68.5%	0.00751	0.1%	--
1,2,3,7,8,9-HxCDF	0.104 *U*	0.185 *U*	0.142 *U*	0.0915 *U*	68.5%	0.00915	0.1%	--
1,2,3,4,6,7,8-HpCDF	0.212 *U*	0.236 *U*	0.246 *U*	0.174 *U*	38.9%	0.00174	0.0%	--
1,2,3,4,7,8,9-HpCDF	0.116 *U*	0.154 *U*	0.236 *U*	0.133 *U*	51.0%	0.00133	0.0%	--
OCDF	0.737 *U*	1.27 *U*	0.577 *U*	0.513 *U*	87.0%	5.13E-05	0.0%	--
TEQ (pg/g)						**11.1**		
PCBs								
PCB-77	--	--	--	3.44	--	0.000344	--	--
PCB-81	--	--	--	2.90 *U*[b]	--	0.00029	--	--
PCB-105	--	--	--	31.1	--	0.00311	--	--
PCB-114	--	--	--	2.90 *U*[b]	--	0.00145	--	--
PCB-106/118	--	--	--	91.6	--	0.00916	--	--
PCB-123	--	--	--	2.90 *U*[b]	--	0.00029	--	--
PCB-126	--	--	--	2.90 *U*[b]	--	0.290	--	--
PCB-156	--	--	--	10.8	--	0.0054	--	--
PCB-157	--	--	--	3.07	--	0.00154	--	--
PCB-167	--	--	--	5.50	--	0.000055	--	--
PCB-169	--	--	--	2.90 *U*[b]	--	0.029	--	--
PCB-189	--	--	--	2.90 *U*[b]	--	0.00029	--	--
TEQ (pg/g)						**0.34**		
Total TEQ (pg/g)						**11.5**		

Note: *B* – This compound was also detected in the method blank.
D – The amount reported is the maximum possible concentration due to possible chlorinated diphenylether interference.
J – The amount detected is below the Lower Calibration Limit of the instrument.
U – Not detected; value represents the sample-specific detection limit, unless noted otherwise.
TEQ – Toxicity Equivalence Concentration
WHO TEF – World Health Organization Toxicity Equivalence Factor
Highlighting indicates the five congeners in each sample that contribute most to the total TEQ
If more than half of the results for a chemical were qualified with a *B*, *D*, or *J*, then the associated mean concentration was also qualifi

[a] Nondetect reported to the EMPC (Estimated Maximum Possible Concentration).
[b] Nondetect reported to the reporting limit.

Table 5. Analytical results for reference mixtures used in rat study

Compound	Initial Concentration (µg/mL)	Amount Spiked (µg/L)	Target Concentration (ng/mL)	Measured Concentration, Pre-Dosing (ng/mL)	Relative Percent Difference[a]	Measured Concentration, Post-Dosing (ng/mL)	Average Measured Concentration[b] (ng/mL)	Coefficient of Variability[c] (%)
Acetone Reference Mixture								
2,3,7,8-TCDD	--	--	0.625	0.664	6.1%	--	--	--
1,2,3,7,8-PeCDD	--	--	0.318	0.346	8.4%	--	--	--
1,2,3,6,7,8-HxCDD	--	--	0.349	0.492	34%	--	--	--
1,2,3,4,6,7,8-HpCDD	--	--	5.54	6.15	10%	--	--	--
2,3,4,7,8-PeCDD	--	--	0.172	0.178	3.4%	--	--	--
Gavage Reference Mixture No. 1 (Alta ID: 040812A)								
2,3,7,8-TCDD	5.0	60.4	0.151	0.142	6.1%	0.114	0.128	15%
1,2,3,7,8-PeCDD	5.0	30.8	0.077	0.079	2.6%	0.0690	0.0740	10%
1,2,3,6,7,8-HxCDD	5.0	33.8	0.084	0.122	37%	0.0901	0.106	21%
1,2,3,4,6,7,8-HpCDD	5.0	536.6	1.342	1.475	9.4%	1.18	1.33	16%
2,3,4,7,8-PeCDF	5.0	16.6	0.042	0.039	7.4%	0.0404	0.0397	2.5%
Gavage Reference Mixture No. 2 (Alta ID: 040812B)								
2,3,7,8-TCDF	50	98.9	2.473	2.655	7.1%	2.04	2.35	19%
1,2,3,7,8-PeCDF	50	49.5	1.238	1.185	4.4%	1.16	1.17	1.5%
2,3,4,7,8-PeCDF	50	40.6	1.015	0.963	5.3%	0.945	0.954	1.3%
1,2,3,4,7,8-HxCDF	5.0	330.8	0.827	0.806	2.6%	0.809	0.808	0.3%
1,2,3,6,7,8-HxCDF	5.0	75.2	0.188	0.214	13%	0.210	0.212	1.3%

[a] The relative percent difference (RPD) between the target and pre-dosing measured concentrations is calculated as the absolute value of the difference divided by the average of the target and pre-dosing measured concentrations.

[b] Average of pre- and post-dosing measured concentrations.

[c] Coefficient of variability between pre- and post-dosing measured concentrations.

Table 6. Analytical results for reference mixtures used in swine study

Compound	Initial Concentration (µg/mL)	Amount Used (µg/L)	Target Concentration (ng/mL)	Measured Concentration, Pre-Dosing (ng/mL)	Relative Percent Difference[a]	Measured Concentration, Post-Dosing (ng/mL)	Average Measured Concentration[b] (ng/mL)	Coefficient of Variability[c] (%)
Swine Reference Oil Mixture No. 1 (Alta ID: 040922A)								
2,3,7,8-TCDD	5.0	131.40	0.328	0.332	1.2%	0.446	0.389	21%
1,2,3,7,8-PeCDD	5.0	66.80	0.167	0.145	14%	0.208	0.177	25%
1,2,3,6,7,8-HxCDD	5.0	73.60	0.184	0.194	5.3%	0.270	0.232	23%
1,2,3,4,6,7,8-HpCDD	50	116.66	2.916	2.385	20%	3.58	2.98	28%
2,3,4,7,8-PeCDF	5.0	36.00	0.090	0.0840	6.9%	0.112	0.0980	20%
Swine Reference Oil Mixture No. 2 (Alta ID:040922B)								
2,3,7,8-TCDF	50	215.00	5.375	4.36	21%	5.44	4.90	16%
1,2,3,7,8-PeCDF	50	107.60	2.690	2.63	2.3%	3.24	2.94	15%
2,3,4,7,8-PeCDF	50	88.26	2.206	2.26	2.2%	2.75	2.50	14%
1,2,3,4,7,8-HxCDF	50	71.94	1.798	1.86	3.1%	2.12	1.99	9.4%
1,2,3,6,7,8-HxCDF	50	16.36	0.409	0.452	10%	0.528	0.490	11%

[a] The relative percent difference (RPD) between the target and pre-dosing measured concentrations is calculated as the absolute value of the difference divided by the average of the target and pre-dosing measured concentrations.

[b] Average of pre- and post-dosing measured concentrations.

[c] Coefficient of variability between pre- and post-dosing measured concentrations.

Table 7. Dose groups and test materials used in the rat pilot study

Dose Group	Test Material Name/ID	Description
1	Gavage Reference Mixture No. 1 (Alta ID: 040812A)	Oral gavage (Midland soil match in corn oil/acetone)
2	Gavage Reference Mixture No. 2 (Alta ID: 040812B)	Oral gavage (Tittabawassee River flood plain soil match in corn oil/acetone)
3	Test Article #1 (soil CC-S-27 in diet)	Midland soil blended with diet
4	Test Article #2 (soil THT02769 in diet)	Tittabawassee River flood plain soil blended with diet
5	Test Article #3 (acetone reference mixture 040728A in diet)	Feed control (Midland soil reference mixture blended with diet)

Table 8. Dose groups and test materials used in the swine pilot study

Dose Group	Test Material Name/ID	Description
1	Swine Reference Mixture No. 1 (Alta ID: 040922A)	Corn oil/acetone in gel capsules (4 mL/day)
2	Swine Reference Mixture No. 2 (Alta ID: 040922B)	Corn oil/acetone in gel capsules (4 mL /day)
3	Midland Soil (CC-S-27)	Midland soil (10 g/day)
4	Tittibawassee River flood plain soil (THT02769)	Tittibawassee River flood plain soil (10 g/day)

Table 9. Average daily doses administered to rats

	WHO TEF	Number of Animals per Group	Soil/Feed Mixture Average Daily Dose (ng/kg bw/day) Mean	S.D.	TEQ	Reference Corn Oil Gavage Average Daily Dose (ng/kg bw/day) Mean	S.D.	TEQ	Reference Feed Average Daily Dose (ng/kg bw/day) Mean	S.D.	TEQ
Midland Soil		10[a]									
2,3,7,8-TCDD	1		0.302	0.017	0.302	0.511	0.014	0.511	0.352	0.024	0.352
1,2,3,7,8-PeCDD	1		0.172	0.0096	0.172	0.295	0.0081	0.295	0.222	0.015	0.222
1,2,3,6,7,8-HxCDD	0.1		0.247	0.014	0.0247	0.423	0.012	0.0423	0.285	0.019	0.0285
1,2,3,4,6,7,8-HpCDD	0.01		4.82	0.27	0.0482	5.31	0.14	0.0531	3.53	0.24	0.0353
2,3,4,7,8-PeCDF	0.5		0.100	0.0056	0.0498	0.158	0.0043	0.0792	0.121	0.0081	0.0607
Total Mean TEQ Dose:			--	--	**0.597**	--	--	**0.981**	--	--	**0.699**
Tittabawassee River Flood Plain Soil		10[a,b]									
2,3,7,8-TCDF	0.1		6.43	0.37	0.643	8.84	1.7	0.884	--	--	--
1,2,3,7,8-PeCDF	0.05		3.92	0.23	0.196	4.40	0.84	0.220	--	--	--
2,3,4,7,8-PeCDF	0.5		3.37	0.20	1.69	3.59	0.68	1.79	--	--	--
1,2,3,4,7,8-HxCDF	0.01		2.63	0.15	0.0263	3.04	0.58	0.0304	--	--	--
1,2,3,6,7,8-HxCDF	0.01		0.649	0.038	0.0065	0.798	0.15	0.0080	--	--	--
Total Mean TEQ Dose:			--	--	**2.56**	--	--	**2.94**	--	--	--

Notes:

WHO TEF – World Health Organization Toxicity Equivalence Factor
S.D. – Standard deviation
TEQ – Toxicity Equivalence Concentration

[a] Tissue samples from rats were grouped into pairs for each analysis to acheive adequate sample mass, resulting in a sample size of 5 for each tissue analysis.

[b] Two rats from the Tittabawassee River flood plain soil corn oil gavage reference group (Group 2) died early and were excluded from calculations of average daily dose and RBA estimates.

Table 10. Summary of EROD and MROD liver microsomal activity data

		Liver Microsomal Activities (pmol/mg/min)				
	N	Minimum	Maximum	Mean	S.D.	p-value[a]
Rat						
EROD						
Midland Soil (Group 3)	5	63	99	83	14	--
Midland Reference Oil (Group 1)	5	116	257	169	53	**0.0194**
Midland Reference Feed (Group 5)	5	121	153	140	15	**0.0002**
Tittabawassee River Flood Plain Soil (Group 4)	5	261	361	319	39	--
Tittabawassee River Flood Plain Soil Reference Oil (Group 2)	4	407	486	444	34	**0.0015**
MROD						
Midland Soil (Group 3)	5	81	120	101	16	--
Midland Reference Oil (Group 1)	5	95	121	108	9.2	0.4006
Midland Reference Feed (Group 5)	5	96	139	122	17	0.0824
Tittabawassee River Flood Plain Soil (Group 4)	5	139	198	168	28	--
Tittabawassee River Flood Plain Soil Reference Oil (Group 2)	4	69	209	163	64	0.8779
Swine						
EROD						
Midland Soil (Group 3)	5	20	27	25	3	--
Midland Reference Oil (Group 1)	5	4	44	25	16	0.9567
Tittabawassee River Flood Plain Soil (Group 4)	4	15	47	28	14	--
Tittabawassee River Flood Plain Soil Reference Oil (Group 2)	5	32	39	35	3.1	0.3729
MROD						
Midland Soil (Group 3)	5	84	138	114	24	--
Midland Reference Oil (Group 1)	5	40	148	95	53	0.4867
Tittabawassee River Flood Plain Soil (Group 4)	4	82	131	97	23	--
Tittabawassee River Flood Plain Soil Reference Oil (Group 2)	5	84	169	123	39	0.2779

Notes: EROD – ethoxyresorufin O-deethylase
MROD – methoxyresorufin O-deethylase
S.D. – standard deviation

[a] Reference groups compared to corresponding soil groups using standard t-tests; p-values reported are unadjusted. Bolded values indicate a significant difference. Comparisons using Wilcox non-parametric test provided identical conclusions.

Table 11. Sensitivity of analytical limits for the rat pilot study

Dosing Group/ Chemical	Liver Number of Analyses	Liver Results Below DL (U)	Liver Results Below EMPC (Um)	Liver Results Below LCL (J)	Adipose Number of Analyses	Adipose Results Below DL (U)	Adipose Results Below EMPC (Um)	Adipose Results Below LCL (J)
Midland Soil (Group 3)								
2,3,7,8-TCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
1,2,3,6,7,8-HxCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
1,2,3,4,6,7,8-HpCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
All chemicals	**25**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**25**	**0 (0%)**	**0 (0%)**	**15 (60%)**
Midland Gavage Oil Reference (Group 1)								
2,3,7,8-TCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,4,6,7,8-HpCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
All chemicals	**25**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**25**	**0 (0%)**	**0 (0%)**	**5 (20%)**
Midland Soil Reference (Group 5)								
2,3,7,8-TCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	4 (80%)
1,2,3,4,6,7,8-HpCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
All chemicals	**25**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**25**	**0 (0%)**	**0 (0%)**	**9 (36%)**
Tittabawassee River Flood Plain Soil (Group 4)								
2,3,7,8-TCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,4,7,8-HxCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	5 (100%)
All chemicals	**25**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**25**	**0 (0%)**	**0 (0%)**	**5 (20%)**
Tittabawassee River Flood Plain Soil Reference (Group 2)[a]								
2,3,7,8-TCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
1,2,3,4,7,8-HxCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
All chemicals	**20**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**20**	**0 (0%)**	**0 (0%)**	**0 (0%)**

Notes:

DL – detection limit (sample specific)
EMPC – estimated maximum possible concentration
LCL – lower calibration limit of the analytical instrument
U – not detected at the sample-specific detection limit
Um – not detected at the EMPC
J – amount detected is below the LCL

[a] Summary values exclude results for the pair of rats that died before the end of the study (Rats #24 and 29).

Table 12. Summary of relative bioavailability estimates for the rat study

	Fraction of Administered Dose Retained									RBA Estimates					
	Liver			Adipose			Liver + Adipose			Liver		Adipose		Liver + Adipose	
Analyte	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
Midland Soil (Group 3)															
2,3,7,8-TCDD	0.042	0.003	7%	0.120	0.016	14%	0.162	0.017	11%						
1,2,3,7,8-PeCDD	0.093	0.006	7%	0.113	0.016	14%	0.206	0.016	8%						
1,2,3,6,7,8-HxCDD	0.166	0.012	7%	0.065	0.008	12%	0.230	0.016	7%						
1,2,3,4,6,7,8-HpCDD	0.089	0.006	7%	0.015	0.002	13%	0.104	0.007	6%						
2,3,4,7,8-PeCDF	0.273	0.017	6%	0.042	0.006	15%	0.315	0.018	6%						
Midland Reference Feed (Group 5)										**Soil vs. Reference Feed**					
2,3,7,8-TCDD	0.110	0.012	11%	0.263	0.030	12%	0.373	0.042	11%	38%	13%	46%	18%	43%	16%
1,2,3,7,8-PeCDD	0.191	0.018	9%	0.182	0.022	12%	0.373	0.039	10%	48%	12%	62%	19%	55%	13%
1,2,3,6,7,8-HxCDD	0.279	0.022	8%	0.080	0.014	18%	0.359	0.033	9%	60%	11%	80%	22%	64%	11%
1,2,3,4,6,7,8-HpCDD	0.159	0.012	7%	0.021	0.003	14%	0.180	0.014	8%	56%	10%	72%	19%	58%	10%
2,3,4,7,8-PeCDF	0.560	0.046	8%	0.063	0.006	10%	0.623	0.051	8%	49%	10%	65%	18%	50%	10%
Midland Reference Gavage (Group 1)										**Soil vs. Reference Gavage**					
2,3,7,8-TCDD	0.139	0.009	7%	0.319	0.017	5%	0.458	0.020	4%	30%	9%	38%	15%	35%	12%
1,2,3,7,8-PeCDD	0.265	0.009	3%	0.250	0.016	6%	0.515	0.013	3%	35%	8%	45%	16%	40%	8%
1,2,3,6,7,8-HxCDD	0.376	0.015	4%	0.117	0.011	9%	0.493	0.014	3%	44%	8%	55%	15%	47%	7%
1,2,3,4,6,7,8-HpCDD	0.265	0.009	3%	0.041	0.005	13%	0.306	0.012	4%	34%	7%	36%	18%	34%	8%
2,3,4,7,8-PeCDF	0.710	0.027	4%	0.086	0.008	9%	0.796	0.022	3%	38%	7%	48%	18%	40%	6%
Tittabawassee River Flood Plain Soil (Group 4)															
2,3,7,8-TCDF	0.065	0.006	10%	0.049	0.010	19%	0.114	0.015	13%						
1,2,3,7,8-PeCDF	0.084	0.007	8%	0.032	0.005	15%	0.117	0.010	9%						
2,3,4,7,8-PeCDF	0.394	0.021	5%	0.031	0.004	12%	0.425	0.022	5%						
1,2,3,4,7,8-HxCDF	0.312	0.017	5%	0.029	0.003	9%	0.341	0.017	5%						
1,2,3,6,7,8-HxCDF	0.327	0.022	7%	0.028	0.003	9%	0.355	0.024	7%						
Tittabawassee River Flood Plain Soil Reference Gavage (Group 2)										**Soil vs. Reference Gavage**					
2,3,7,8-TCDF	0.072	0.004	5%	0.055	0.003	5%	0.127	0.006	5%	90%	11%	89%	20%	89%	14%
1,2,3,7,8-PeCDF	0.142	0.008	6%	0.060	0.007	11%	0.202	0.014	7%	59%	10%	54%	19%	58%	11%
2,3,4,7,8-PeCDF	0.750	0.036	5%	0.061	0.007	12%	0.811	0.040	5%	52%	7%	52%	17%	52%	7%
1,2,3,4,7,8-HxCDF	0.545	0.017	3%	0.055	0.008	14%	0.599	0.020	3%	57%	6%	54%	16%	57%	6%
1,2,3,6,7,8-HxCDF	0.582	0.032	6%	0.051	0.007	14%	0.633	0.034	5%	56%	9%	55%	17%	56%	9%

Notes: One outlier excluded from Group 4 for 1,2,3,6,7,8-HxCDF. See text for details

RBA – relative bioavailability, calculated as: Fraction of administered dose retained$_{\text{test material}}$ / Fraction of administered dose retained$_{\text{reference material}}$

S.D. – standard deviation

C.V. – coefficient of variability

For fraction of administered dose retained: C.V. = Standard Deviation / Mean

For RBA estimates: C.V. = $(CV_{soil}^{2} + CV_{reference}^{2})^{0.5}$

Table 13. Average daily doses administered to swine

	WHO TEF	Number of Animals per Group	Soil/Feed Mixture Average Daily Dose (ng/kg bw/day) Mean	S.D.	TEQ	Reference Corn Oil Average Daily Dose (ng/kg bw/day) Mean	S.D.	TEQ
Midland Soil		5						
2,3,7,8-TCDD	1		0.0699	0.0024	0.0699	0.0807	0.0038	0.0807
1,2,3,7,8-PeCDD	1		0.0356	0.0012	0.0356	0.0367	0.0017	0.0367
1,2,3,6,7,8-HxCDD	0.1		0.0391	0.0013	0.0039	0.0482	0.0023	0.0048
1,2,3,4,6,7,8-HpCDD	0.01		0.621	0.021	0.0062	0.619	0.029	0.0062
2,3,4,7,8-PeCDF	0.5		0.0192	0.0006	0.0096	0.0203	0.0010	0.0102
Tittabawassee River Flood Plain Soil		5[a]						
2,3,7,8-TCDF	0.1		1.12	0.045	0.112	1.08	0.036	0.108
1,2,3,7,8-PeCDF	0.05		0.561	0.023	0.0280	0.647	0.021	0.0324
2,3,4,7,8-PeCDF	0.5		0.460	0.018	0.230	0.550	0.018	0.275
1,2,3,4,7,8-HxCDF	0.01		0.375	0.015	0.0038	0.438	0.014	0.0044
1,2,3,6,7,8-HxCDF	0.01		0.0853	0.0034	0.0009	0.108	0.0036	0.0011

Notes:

WHO TEF – World Health Organization Toxicity Equivalence Factor
S.D. – Standard deviation
TEQ – Toxicity Equivalence Concentration

[a] One swine from Group 4 died early and was excluded from calculations of average daily dose and RBA estimates.

Table 14. Sensitivity of analytical limits for the swine pilot study

Dosing Group/ Chemical	Liver				Adipose			
	Number of Analyses	Results Below			Number of Analyses	Results Below		
		DL (U)	EMPC (Um)	LCL (J)		DL (U)	EMPC (Um)	LCL (J)
Midland Soil (Group 3)								
2,3,7,8-TCDD	5	1 (20%)	0 (0%)	4 (80%)	5	0 (0%)	2 (40%)	3 (60%)
1,2,3,7,8-PeCDD	5	3 (60%)	1 (20%)	1 (20%)	5	0 (0%)	3 (60%)	2 (40%)
1,2,3,6,7,8-HxCDD	5	1 (20%)	3 (60%)	1 (20%)	5	1 (20%)	1 (20%)	3 (60%)
1,2,3,4,6,7,8-HpCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	5 (100%)	5	1 (20%)	3 (60%)	1 (20%)
All chemicals	**25**	**5 (20%)**	**4 (16%)**	**11 (44%)**	**25**	**2 (8%)**	**9 (36%)**	**9 (36%)**
Midland Oil Reference (Group 1)								
2,3,7,8-TCDD	5	0 (0%)	1 (20%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDD	5	0 (0%)	2 (40%)	3 (60%)	5	0 (0%)	0 (0%)	5 (100%)
1,2,3,6,7,8-HxCDD	5	0 (0%)	1 (20%)	4 (80%)	5	0 (0%)	0 (0%)	5 (100%)
1,2,3,4,6,7,8-HpCDD	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	5 (100%)	5	0 (0%)	0 (0%)	5 (100%)
All chemicals	**25**	**0 (0%)**	**4 (16%)**	**12 (48%)**	**25**	**0 (0%)**	**0 (0%)**	**15 (60%)**
Tittabawassee River Soil (Group 4)[a]								
2,3,7,8-TCDF	4	4 (100%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	1 (25%)
1,2,3,7,8-PeCDF	4	4 (100%)	0 (0%)	0 (0%)	4	0 (0%)	1 (25%)	3 (75%)
2,3,4,7,8-PeCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
1,2,3,4,7,8-HxCDF	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDF	4	0 (0%)	0 (0%)	2 (50%)	4	0 (0%)	0 (0%)	4 (100%)
All chemicals	**20**	**8 (40%)**	**0 (0%)**	**2 (10%)**	**20**	**0 (0%)**	**1 (5%)**	**8 (40%)**
Tittabawassee River Oil Reference (Group 2)								
2,3,7,8-TCDF	5	0 (0%)	0 (0%)	1 (20%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,7,8-PeCDF	5	1 (20%)	3 (60%)	1 (20%)	5	0 (0%)	0 (0%)	5 (100%)
2,3,4,7,8-PeCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,4,7,8-HxCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	0 (0%)
1,2,3,6,7,8-HxCDF	5	0 (0%)	0 (0%)	0 (0%)	5	0 (0%)	0 (0%)	1 (20%)
All chemicals	**25**	**1 (4%)**	**3 (12%)**	**2 (8%)**	**25**	**0 (0%)**	**0 (0%)**	**6 (24%)**

Notes:

DL – detection limit (sample specific)
EMPC – estimated maximum possible concentration
LCL – lower calibration limit of the analytical instrument
U – not detected at the sample-specific detection limit
Um – not detected at the EMPC
J – amount detected is below the LCL

[a] Summary values exclude results for the swine that died before the end of the study (#444).

Table 15a. Summary of relative bioavailability estimates for the swine study (using 1/2 DL)

	Fraction of Administered Dose Retained									RBA Estimates					
	Liver			Adipose			Liver + Adipose			Liver		Adipose		Liver + Adipose	
Analyte	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
Midland Soil (Group 3)															
2,3,7,8-TCDD	0.0039	0.0014	35%	0.028	0.013	46%	0.032	0.013	41%						
1,2,3,7,8-PeCDD	0.0043	0.0023	55%	0.040	0.018	46%	0.044	0.018	40%						
1,2,3,6,7,8-HxCDD	0.0070	0.0037	54%	0.073	0.042	58%	0.080	0.043	54%						
1,2,3,4,6,7,8-HpCDD	0.0185	0.0063	34%	0.046	0.011	24%	0.064	0.016	24%						
2,3,4,7,8-PeCDF	0.0440	0.0121	27%	0.042	0.024	57%	0.086	0.025	29%						
Midland Reference Oil (Group 1)										**Soil vs. Reference Oil**					
2,3,7,8-TCDD	0.0102	0.0034	33%	0.165	0.016	10%	0.175	0.019	11%	38%	48%	17%	47%	18%	43%
1,2,3,7,8-PeCDD	0.0123	0.0043	35%	0.173	0.020	12%	0.185	0.018	10%	35%	65%	23%	47%	24%	41%
1,2,3,6,7,8-HxCDD	0.0194	0.0057	29%	0.188	0.021	11%	0.208	0.022	11%	36%	61%	39%	59%	38%	55%
1,2,3,4,6,7,8-HpCDD	0.0290	0.0080	27%	0.089	0.018	20%	0.118	0.024	21%	64%	44%	52%	31%	55%	32%
2,3,4,7,8-PeCDF	0.0956	0.0146	15%	0.175	0.016	9%	0.270	0.029	11%	46%	31%	24%	58%	32%	31%
Tittabawassee River Flood Plain Soil (Group 4)															
2,3,7,8-TCDF	1.2E-04	2.9E-05	25%	0.0026	4.8E-04	18%	0.003	4.6E-04	17%						
1,2,3,7,8-PeCDF	2.4E-04	2.7E-05	11%	0.0033	0.0015	45%	0.004	0.0015	42%						
2,3,4,7,8-PeCDF	0.0273	0.0011	4%	0.0419	0.0051	12%	0.069	0.0049	7%						
1,2,3,4,7,8-HxCDF	0.0233	0.0024	10%	0.0675	0.0055	8%	0.091	0.0059	6%						
1,2,3,6,7,8-HxCDF	0.0333	0.0019	6%	0.0646	0.0037	6%	0.098	0.0043	4%						
Tittabawassee River Flood Plain Reference Oil (Group 2)										**Soil vs. Reference Oil**					
2,3,7,8-TCDF	0.0005	1.5E-04	28%	0.0119	0.0024	20%	0.012	0.0024	19%	21%	38%	22%	27%	22%	26%
1,2,3,7,8-PeCDF	0.0003	9.7E-05	34%	0.0117	0.0020	17%	0.012	0.0021	18%	86%	36%	28%	48%	30%	46%
2,3,4,7,8-PeCDF	0.1038	0.0202	19%	0.1499	0.0268	18%	0.254	0.0286	11%	26%	20%	28%	22%	27%	13%
1,2,3,4,7,8-HxCDF	0.0686	0.0135	20%	0.1877	0.0241	13%	0.256	0.0251	10%	34%	22%	36%	15%	35%	12%
1,2,3,6,7,8-HxCDF	0.0951	0.0198	21%	0.1668	0.0209	13%	0.262	0.0206	8%	35%	22%	39%	14%	37%	9%

Notes:

RBA – relative bioavailability adjustment

RBA calculated as: Fraction of administered dose retained$_{\text{test material}}$ / Fraction of administered dose retained$_{\text{reference material}}$

S.D. – standard deviation

C.V. – coefficient of variability

For fraction of administered dose retained: C.V. = Standard Deviation / Mean

For RBA estimates: C.V. = $(CV_{soil}^{2} + CV_{reference}^{2})^{0.5}$

Table 15b. Summary of relative bioavailability estimates for the swine study (using DL)

	Fraction of Administered Dose Retained									RBA Estimates					
	Liver			Adipose			Liver + Adipose			Liver		Adipose		Liver + Adipose	
Analyte	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
Midland Soil (Group 3)															
2,3,7,8-TCDD	0.0042	0.0008	18%	0.034	0.006	18%	0.038	0.006	17%						
1,2,3,7,8-PeCDD	0.0069	0.0014	21%	0.057	0.010	18%	0.064	0.011	17%						
1,2,3,6,7,8-HxCDD	0.0113	0.0027	24%	0.084	0.029	35%	0.095	0.029	30%						
1,2,3,4,6,7,8-HpCDD	0.0185	0.0063	34%	0.046	0.011	24%	0.064	0.016	24%						
2,3,4,7,8-PeCDF	0.0440	0.0121	27%	0.067	0.014	20%	0.111	0.018	16%						
Midland Reference Oil (Group 1)										**Soil vs. Reference Oil**					
2,3,7,8-TCDD	0.0111	0.0016	15%	0.165	0.016	10%	0.176	0.017	10%	38%	23%	20%	20%	22%	20%
1,2,3,7,8-PeCDD	0.0153	0.0004	2%	0.173	0.020	12%	0.188	0.020	11%	45%	21%	33%	22%	34%	20%
1,2,3,6,7,8-HxCDD	0.0215	0.0029	13%	0.188	0.021	11%	0.210	0.023	11%	52%	28%	45%	36%	45%	32%
1,2,3,4,6,7,8-HpCDD	0.0290	0.0080	27%	0.089	0.018	20%	0.118	0.024	21%	64%	44%	52%	31%	55%	32%
2,3,4,7,8-PeCDF	0.0956	0.0146	15%	0.175	0.016	9%	0.270	0.029	11%	46%	31%	39%	22%	41%	19%
Tittabawassee River Flood Plain Soil (Group 4)															
2,3,7,8-TCDF	2.3E-04	5.8E-05	25%	0.0026	4.8E-04	18%	0.003	4.5E-04	16%						
1,2,3,7,8-PeCDF	4.9E-04	5.4E-05	11%	0.0036	0.0010	26%	0.004	0.0010	24%						
2,3,4,7,8-PeCDF	0.0273	0.0011	4%	0.0419	0.0051	12%	0.069	0.0049	7%						
1,2,3,4,7,8-HxCDF	0.0233	0.0024	10%	0.0675	0.0055	8%	0.091	0.0059	6%						
1,2,3,6,7,8-HxCDF	0.0333	0.0019	6%	0.0646	0.0037	6%	0.098	0.0043	4%						
Tittabawassee River Flood Plain Reference Oil (Group 2)										**Soil vs. Reference Oil**					
2,3,7,8-TCDF	0.0005	1.5E-04	28%	0.0119	0.0024	20%	0.012	0.0024	19%	42%	38%	22%	27%	23%	25%
1,2,3,7,8-PeCDF	0.0005	9.2E-05	19%	0.0117	0.0020	17%	0.012	0.0020	17%	102%	22%	31%	31%	34%	29%
2,3,4,7,8-PeCDF	0.1038	0.0202	19%	0.1499	0.0268	18%	0.254	0.0286	11%	26%	20%	28%	22%	27%	13%
1,2,3,4,7,8-HxCDF	0.0686	0.0135	20%	0.1877	0.0241	13%	0.256	0.0251	10%	34%	22%	36%	15%	35%	12%
1,2,3,6,7,8-HxCDF	0.0951	0.0198	21%	0.1668	0.0209	13%	0.262	0.0206	8%	35%	22%	39%	14%	37%	9%

Notes:

RBA – relative bioavailability adjustment

RBA calculated as: Fraction of administered dose retained$_{\text{test material}}$ / Fraction of administered dose retained$_{\text{reference material}}$

S.D. – standard deviation

C.V. – coefficient of variability

For fraction of administered dose retained: C.V. = Standard Deviation / Mean

For RBA estimates: C.V. = $(CV_{soil}^{2} + CV_{reference}^{2})^{0.5}$

Table 16. TEQ-weighted relative and absolute bioavailability estimates for two soils

Congener	Percent of Soil TEQ	Mean RBA[a]			Estimated Absolute Bioavailability[b]			Estimated Bioaccessibility[c] (*in vitro* assay)
		Rat	Swine ND=1/2 DL	Swine ND=DL	Rat	Swine ND=1/2 DL	Swine ND=DL	
Midland Soil								
2,3,7,8-TCDD	48.9%	0.35	0.18	0.22	0.28	0.15	0.18	0.17
1,2,3,7,8-PeCDD	24.9%	0.40	0.24	0.34	0.32	0.19	0.27	0.16
1,2,3,6,7,8-HxCDD	2.7%	0.47	0.38	0.45	0.37	0.31	0.36	0.18
1,2,3,4,6,7,8-HpCDD	4.3%	0.34	0.55	0.55	0.27	0.44	0.44	0.26
2,3,4,7,8-PeCDF	6.7%	0.40	0.32	0.41	0.32	0.25	0.33	0.18
TEQ-Weighted:		**0.37**	**0.23**	**0.29**	**0.30**	**0.19**	**0.23**	**0.17**
Tittabawassee River Flood Plain Soil								
2,3,7,8-TCDF	25.4%	0.89	0.22	0.23	0.72	0.18	0.18	--
1,2,3,7,8-PeCDF	6.3%	0.58	0.30	0.34	0.46	0.24	0.27	--
2,3,4,7,8-PeCDF	52.1%	0.52	0.27	0.27	0.42	0.22	0.22	--
1,2,3,4,7,8-HxCDF	8.5%	0.57	0.35	0.35	0.46	0.28	0.28	--
1,2,3,6,7,8-HxCDF[d]	1.9%	0.56	0.37	0.37	0.45	0.30	0.30	--
TEQ-Weighted:		**0.63**	**0.27**	**0.27**	**0.51**	**0.22**	**0.22**	--

[a] RBA estimates for soil compared to corn oil reference material based on liver plus adipose tissue measurements.

[b] Assuming an absolute availability from corn oil of 80%.

[c] As estimated for the Midland soil sample based on in vitro assay by Ruby et al. (2002)

[d] Outlier omitted from rat RBA estimate; see results section text for discussion.

Appendix A

Sampling and Analysis Plan—Soil Sampling for the Pilot Bioavailability Study

Final

Sampling and Analysis Plan
Soil Sampling for Pilot Bioavailability Study

Prepared for

The Dow Chemical Company

June 2004

CH2MHILL

Contents

Tables **Page**

Figures

Abbreviations and Acronyms

ATV	all-terrain vehicle
bgs	below ground surface
COC	chain of custody
Dow	The Dow Chemical Company
DPT	direct push technology
D&F	dioxins and furans
DQO	data quality objective
GIS	geographic information system
GPS	global positioning system
HS&E	Health, Safety, and Environment
HSP	health and safety plan
ID	identification
JHA	job hazard analysis
LTI	Limno-Tech Inc.
MDEQ	Michigan Department of Environmental Quality
MI-OSHA	Michigan Occupational Safety and Health Administration
MOCA	Midland Offsite Corrective Actions
MS/MSD	matrix spike/matrix spike duplicate
PCOI	potential contaminants of interest
ppt	part per trillion
QAPP	quality assurance project plan
RI	remedial investigation
SAP	sampling and analysis plan
site	Tittabawassee River study area
SOP	standard operating procedure
STAC	Safety Task Analysis Card
SWP	Safety Work Permit
USEPA	United States Environmental Protection Agency

1 Introduction

1.1 Background

Several previous investigations, conducted by the Michigan Department of Environmental Quality (MDEQ), have indicated that dioxins and furans may be present in sediment and soil of the Tittabawassee River and its floodplain. On June 12, 2003, MDEQ issued an Operating License to The Dow Chemical Company (Dow). A pilot bioavailability study is being performed to evaluate a study design to assess the oral absorption of dioxins and furans in Midland and the Tittabawassee River floodplain,. This SAP is being prepared for the collection of soil samples from areas within Midland and the Tittabawassee River Floodplain that may be used in the pilot bioavailability study.

1.2 Purpose and Objectives

The purpose and primary objective of this Sampling and Analysis Plan (SAP) is to collect surface soil samples that may be used in the Pilot Bioavailability Study. Samples will be collected in areas where previous sampling results have indicated that dioxins and furans may be present in the concentration range of 800 to 1,000 ppt TEQ.

1.3 Scope

The scope of the field effort described in this SAP includes surface soil sample collection within the Midland area and the Tittabawassee River Floodplain, refer to Figure 1-1. Exponent will coordinate the analysis of all samples collected during this SAP.

Sampling will be performed in accordance with the Field SOPs established for the Dow Midland Off-site Corrective Actions (MOCA) program, and the Dow MOCA *Quality Assurance Project Plan* (QAPP) (CH2M HILL 2004c).

1.4 Data Quality Objectives

Data quality objectives (DQOs) are both qualitative and quantitative statements that define the type, quality, and quantity of data necessary to support the decision making process during project activities. The DQO process used for this project follows the USEPA *Guidance for the Data Quality Objectives Process (EPA QA/G-4)* document (USEPA, 2000) and uses the seven-step DQO development process identified in the QAPP. Table 1-1 presents the DQOs associated with the sampling activities in support of the pilot bioavailability study.

1.5 Project Team

The team members responsible for the effective execution of this SAP are identified by role in Table 1-2. The program management roles are further defined in the Dow MOCA *Program Management Plan* (CH2M HILL, 2004a).

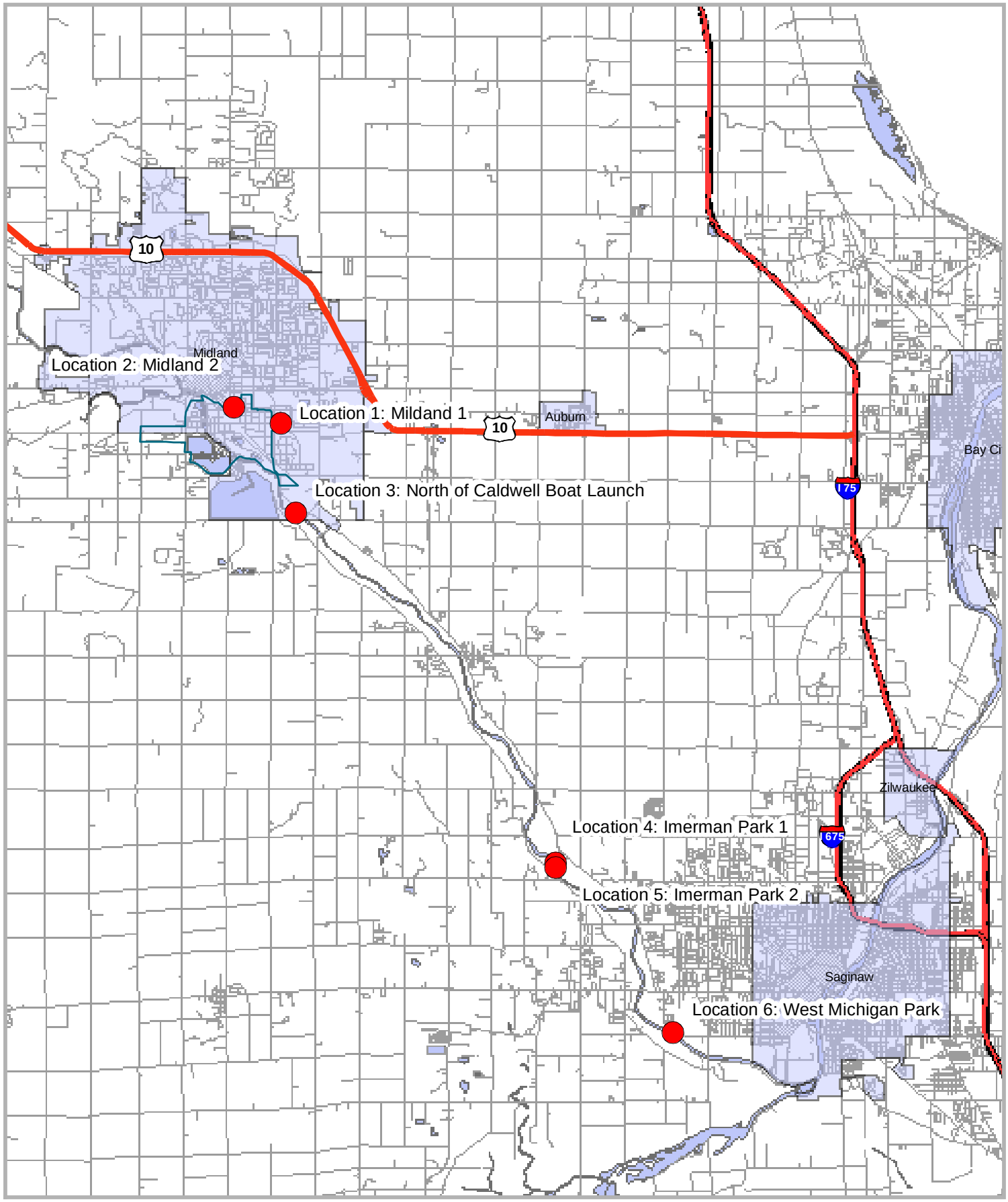

FIGURE 1-1
General Location of Sampling Points
Sampling and Analysis Plan for Ecological Risk Assessment Support Sampling
Dow Midland Offsite Corrective Actions Program

CH2MHILL

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TABLE 1-1
Data Quality Objectives
Pilot Bioavailability Study Support Sampling

State the Problem	Identify the Decisions	Identify Inputs to the Decisions	Define the Boundaries to the Study	Develop a Decision Rule	Specify Tolerable Limits on Decision Errors	Optimize the Design for Obtaining Data
Soil needs to be obtained with concentrations ideally ranging from 800 to 1,000 ppt TEQ of dioxins and furans (D&F) for the pilot bioavailability study.	What locations are likely to have D&F concentrations in the range needed for the pilot bioavailability study?	Surface soils from 0-0.1 ft. Midland area and 0-0.5ft the Titttabawassee River Floodplain.	Surface soils in Midland area and the Tittabawassee River Floodplain with expected D&F TEQ concentrations in the range of 800 to 1,000 ppt TEQ.	If the collected samples do not meet the requirements of Exponent, then additional samples may be collected.	Exponent will determine the tolerable limits on decision errors. Standard operating procedures for soil sampling will be followed to minimize human error.	One to two samples will be collected in the Midland area, and three to four samples will be collected in the Tittabawassee River Floodplain. These locations will be accessed through Dow-owned parcels or via public areas. A minimum of three gallons of soil will be collected per sample.

TABLE 1-2
ERA Support Sampling Project Team
Bioavailability Study Support Sampling

Responsibility	Individual	Affiliation	Contact Information
Senior Environmental Project Leader	Ben Baker	Dow	47 Building Midland, MI 48667 (989) 636-0787
Project Manager Leader/ Client Point-of-Contact	Gary Dyke	CH2M HILL	1111 Washington Street Midland, MI 48640 (989) 835-1187
Pilot Bioavailability Study	Mike Ruby	Exponent	(303) 444-7270
Project Manager	Eric Kroger	CH2M HILL	(937) 228-3180, ext. 207
Field Team Leader	Paul Arps	CH2M HILL	1111 Washington Street Midland, MI 48640 (989) 835-5132
Field Lead	Wayne Ekren	CH2M HILL	(517) 347-3138, ext.42
MOCA Health and Safety Manager	Lisa Martin	CH2M HILL	(816) 224-6311
GIS Manager	Randy Vanslambrouck	CH2M HILL	1111 Washington Street Midland, MI 48640 (989) 832-2608
Data Manager	Linda Crownover	CH2M HILL	(215) 563-4244, ext. 448
Project Chemist	Herb Kelly	CH2M HILL	(352) 335-5877, ext. 2572

2 Field Activities

The following provides some information necessary for the field team to locate the pre-selected sample areas. Each sample location was selected based on previous analytical data.

The soil sample locations will be on either Dow-owned property or in public parks. Access to the public parks will require access agreements. The sample locations are presented in Figure (2-1 through 2-5)

2.1 Access to Surface Soil Sample Locations

Before initiating fieldwork, the appropriate notifications must be made with the property owner at each location. Before entering Dow-owned property, contact Dow Midland Security (refer to Table 2.1). Additionally, the field lead should notify the property owner of the sampling activities the day before they are to commence.

2.1.1 Utility Clearances

Utility clearances are not necessary for the collection of shallow surface soil samples. However, if deemed necessary, the following service is available for identifying and locating underground utilities in Michigan:

Miss Dig System, Inc.
1-800-482-7171

The Miss Dig System should be contacted at least 3 business days prior to beginning any work requiring utility clearances. If questions arise in the field regarding utility clearances, the numbers of each utility owner are included in the Dow MOCA Program Health, Safety and Environment (HS&E) Plan (CH2M HILL, 2003).

2.1.2 Access Agreements

Imerman Park and West Michigan Park require access agreements in order to conduct the surface soil sampling. Access agreements will be secured at these two locations prior to sampling.

2.2 Sampling Procedures

Soil Sampling

Locate the sampling area in the field and verify the location by global positioning system (GPS). Figures 2-1 through 2-5 illustrate the sample locations.

After identifying the sampling location, vegetation/debris will be removed from the surface, taking care not to disturb underlying soil (refer to *Manual Soil Sampling Field SOP 2.1* [CH2M HILL, 2004b]). Only the top 0.1-ft of surface soil will be collected in the Midland area and the

top 0.5-ft will be collected in the Tittabawassee River Floodplain. The sample will be classified using the applicable portions of the *Soil Classification and Logging SOP 2.7*. The sample will be collected into the sample container (3 or 5 gallon bucket).

After collecting enough soil to meet the three-gallon requirement, GPS coordinates will be recorded from each location and documented in the field logbook. The sample location will also be photographed in accordance with the *Digital Camera Use and Documentation Procedures SOP 7.1*. Site restoration will consist of ground cover being placed over the sample location, returning it to its native condition.

2.3 Sample Containers, Preservation, and Holding Times

New 3 or 5 gallon plastic paint buckets will be used to contain the surface soil samples.

The activities associated with the sampling activities must be documented in field logbooks. The procedures and QC procedures for field logbook entries are located in the *Field SOPs* (CH2M HILL, 2004b) and QAPP (CH2M HILL, 2004c).

2.4 Field Quality Control

Field quality control sample collection is not necessary for this field event.FiF

2.5 Sample Identification

Sample identification numbers are listed in Appendix A (refer to *the Sample Identification Technical Memorandum*, CH2M HILL, 2004e).

2.6 Sample Handling and Chain of Custody

The procedures used for proper packaging, shipping, and documentation of samples being transported from the field to the Exponent for analysis are given in the *Sample Handling and Shipping Custody Procedures Field SOP 6.2* (CH2M HILL, 2004b). Due to the nature and use of the sample, the containers will not be placed on ice for shipping.

After samples are labeled and packaged, they will be shipped to Exponent, at the following address:

Attn: Mike Ruby
Exponent
4940 Pearl East Circle, Suite 300
Boulder, CO 80301
(303) 444-7270

2.7 Equipment Decontamination

- Personal decontamination procedures followed will be those provided in the Dow Program CH2M HILL Health, Safety and Environment Plan (HSEP; CH2M HILL, 2004).
- All soil sampling equipment will be decontaminated in accordance with the *Field Decontamination Procedures Field SOP* (CH2M HILL, 2004b).
- Excess soil, disposable sampling equipment, and decontamination materials and liquids will be disposed of in accordance to the *Handling and Disposal of Investigative-Derived Waste Field SOP* (CH2M HILL. 2004b).

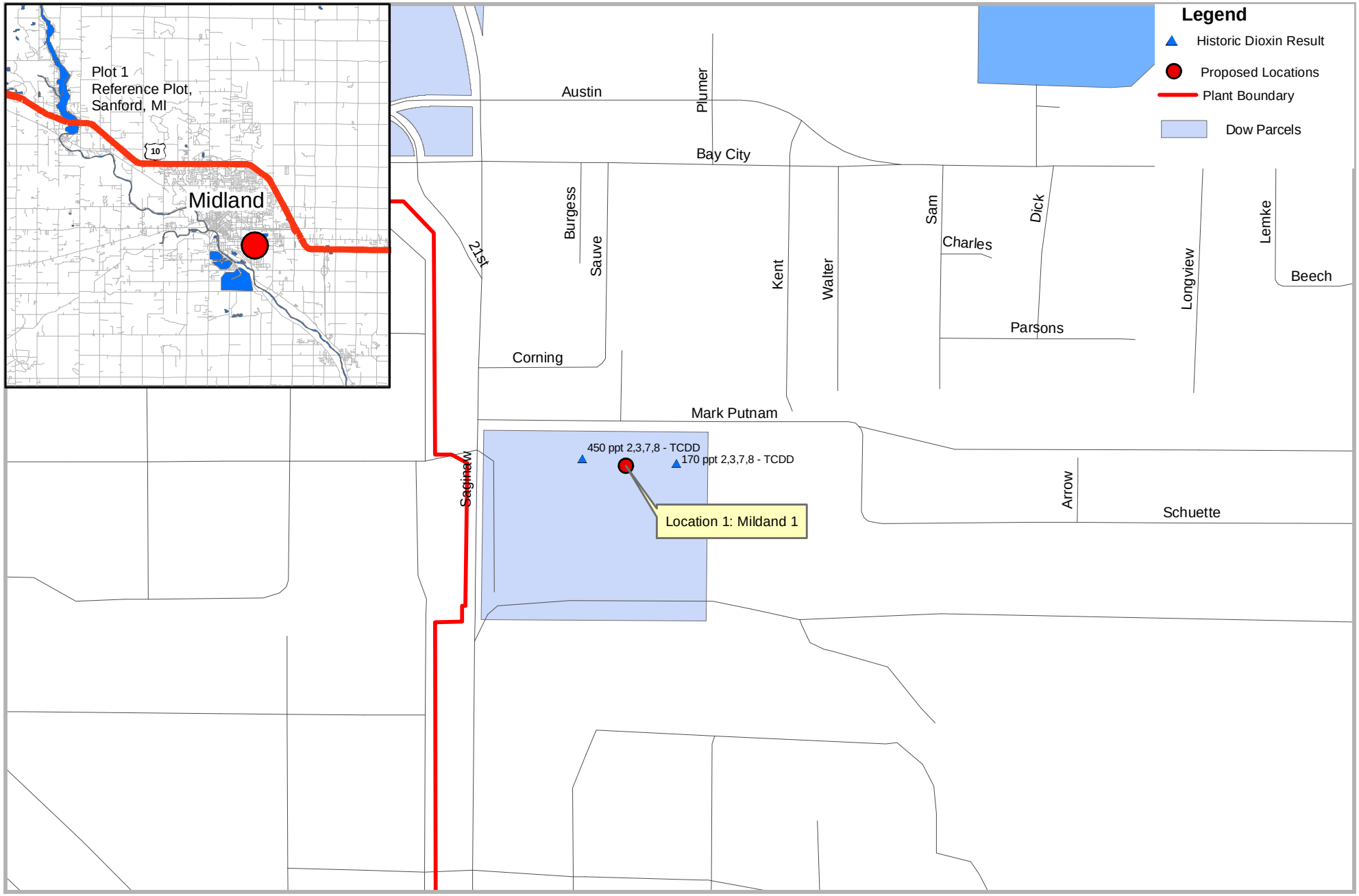

FIGURE 2-1
Location 1
Midland 1 - East of Plant
Sampling and Analysis Plan for Bioavailability Study Support Sampling
Dow Midland Offsite Corrective Actions Program

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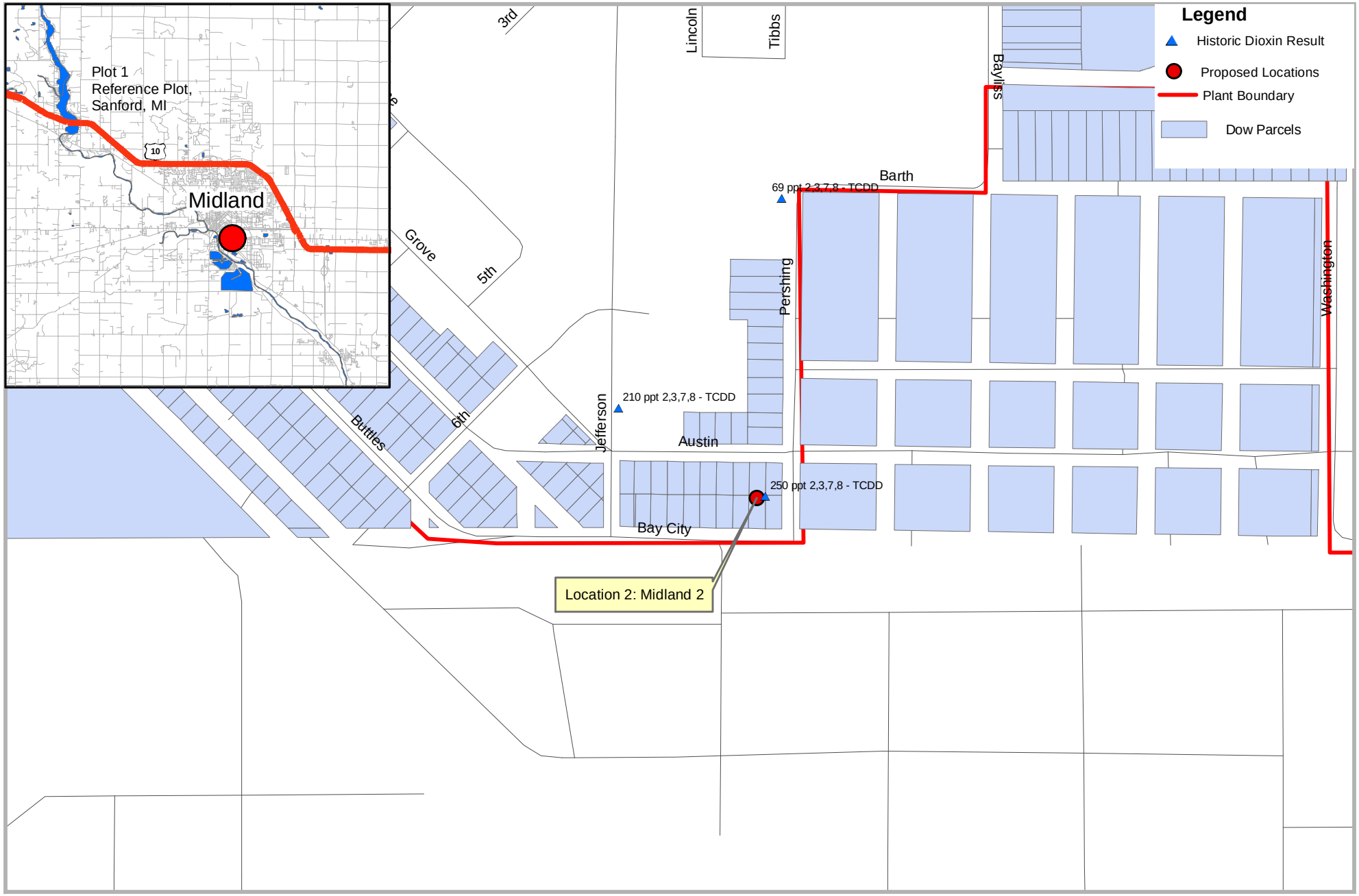

FIGURE 2-2
Location 2
Midland 2 - North of Plant
Sampling and Analysis Plan for Bioavailability Study Support Sampling
Dow Midland Offsite Corrective Actions Program

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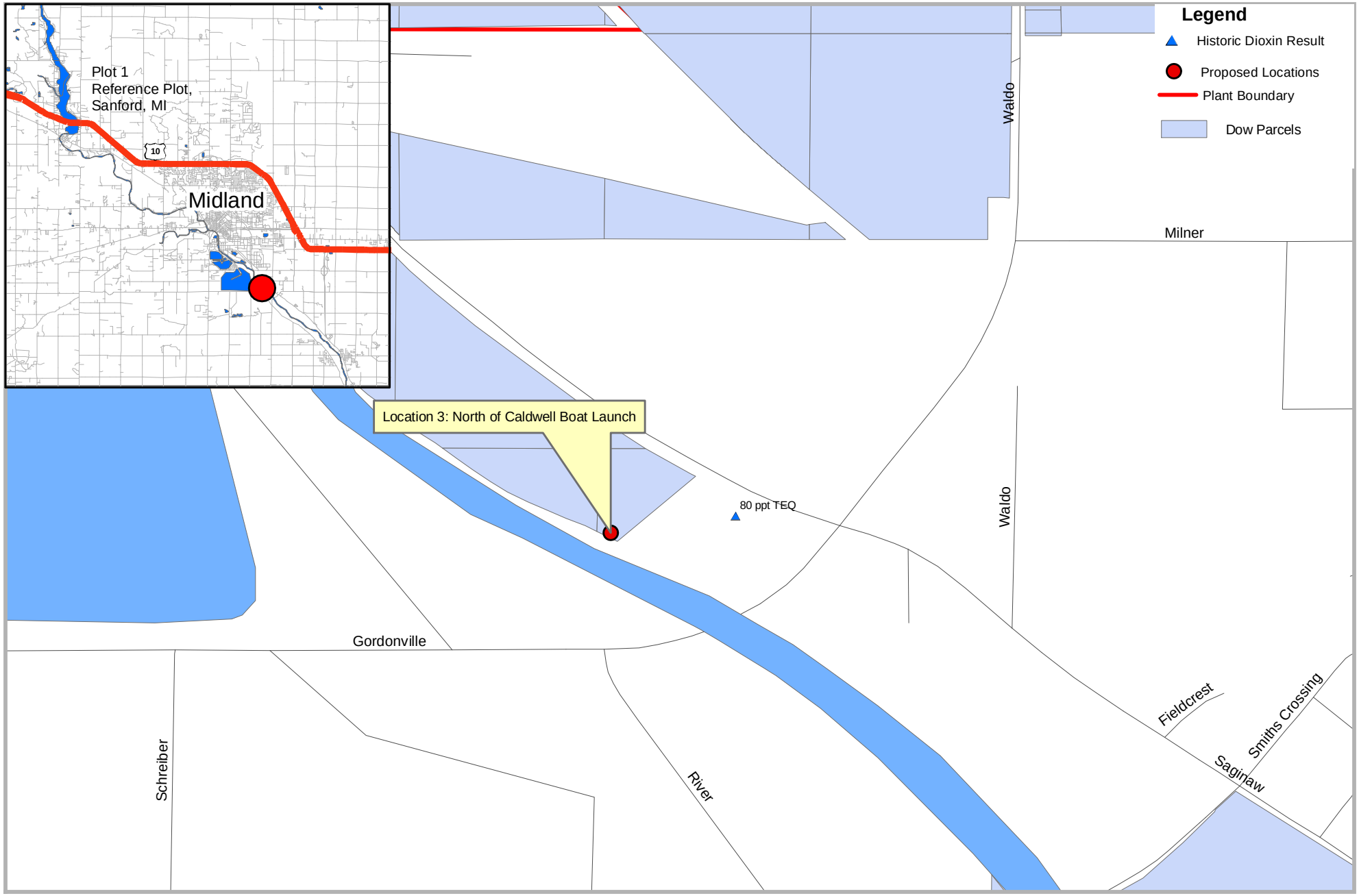

FIGURE 2-3
Location 3
North of Caldwell Boat Launch
Sampling and Analysis Plan for Bioavailability Study Support Sampling
Dow Midland Offsite Corrective Actions Program

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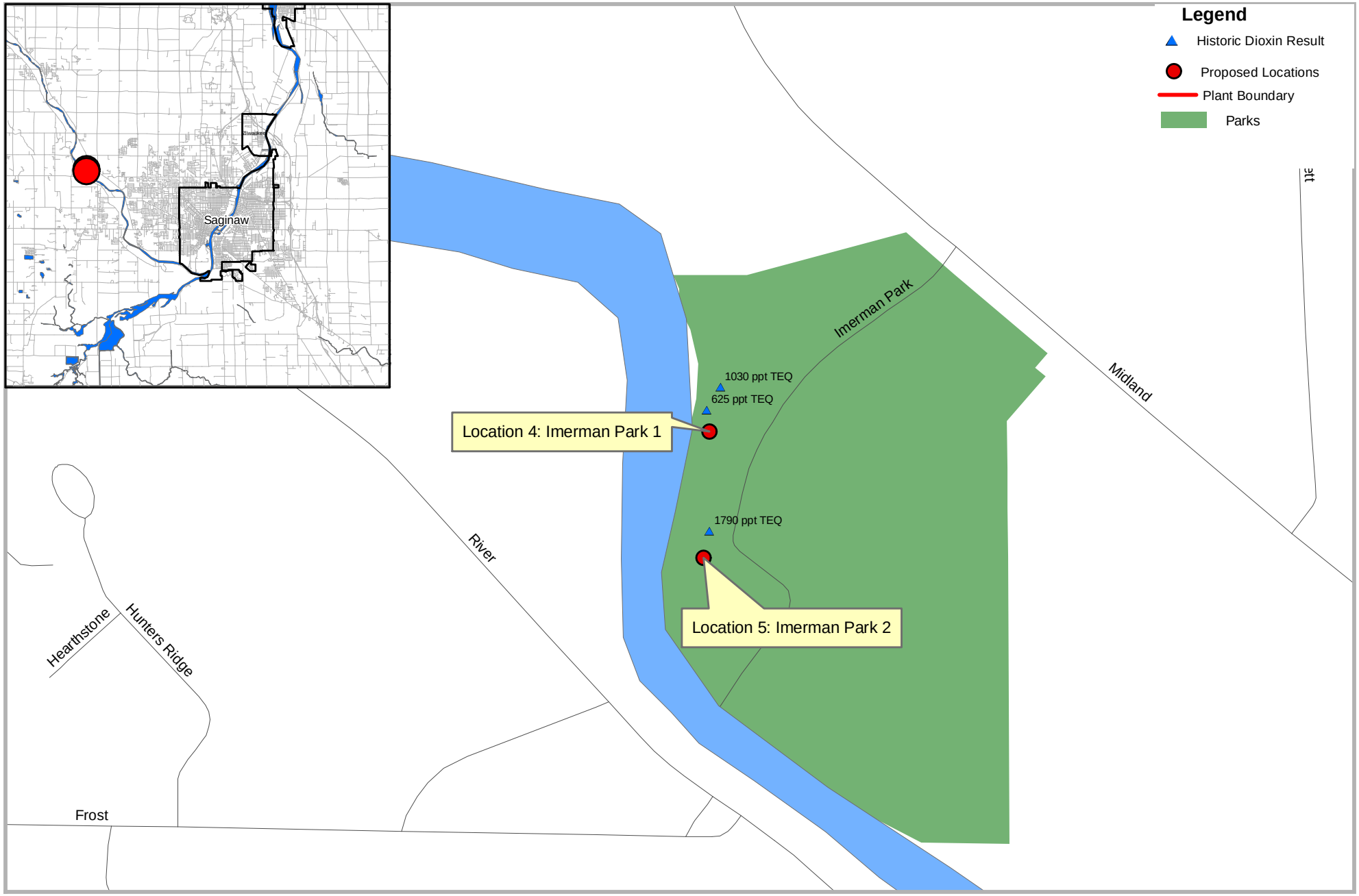

FIGURE 2-4
Location 4 & 5
Imerman Park 1 & Imerman Park 2
Sampling and Analysis Plan for Bioavailability Study Support Sampling
Dow Midland Offsite Corrective Actions Program

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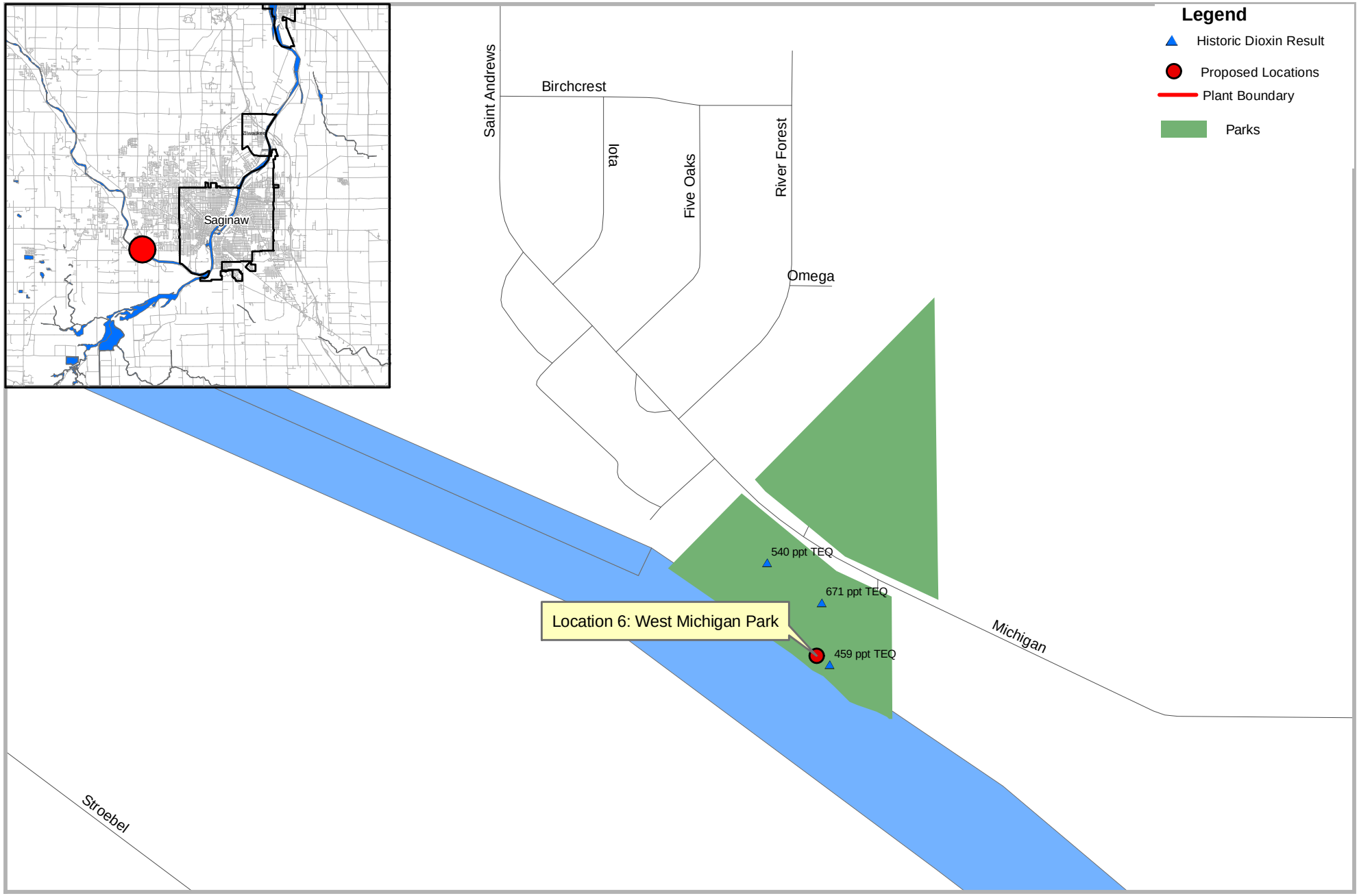

FIGURE 2-5
Location 6
West Michigan Park
Sampling and Analysis Plan for Bioavailability Study Support Sampling
Dow Midland Offsite Corrective Actions Program

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3 Data Management and Validation

All data collected under this field effort will be managed in accordance with the Data Management Plan for Dow MOCA (CH2M HILL, 2004d).

Data validation is not anticipated as part of the data collection process. However if data validation is deemed necessary, all validation will be performed in accordance to the Dow MOCA program QAPP.

4 Health and Safety

Site Specific HS&E Plan Amendment

A Site-Specific Amendment to the HS&E Plan has been prepared for this project and has been approved by The Health and Safety Manager (HSM). It is included with this SAP as Appendix C. Prior to beginning fieldwork, Field Team members must read and sign the amendment, and follow its requirements.

5 Project Schedule

The surface soil collection is scheduled for June 18th. Based on that start date, the schedule will be as follows:

Activity	Anticipated Duration	Anticipated Start Date	Anticipated End Date
Work Planning, SAP Development, Contractor Procurement, Access Agreements	4 Days	June 14, 2004	June 17, 2004
Soil Sampling	1 Days	June 18, 2004	June 18, 2004

6 References

CH2M HILL. 2004. Dow Program CH2M HILL Health, Safety and Environment Plan. December

CH2M HILL. 2004a. *Dow MOCA Program Management Plan.*

CH2M HILL. 2004b. *Field SOPs.* April

CH2M HILL. 2004c. *Quality Assurance Project Plan (QAPP).* April

CH2M HILL. 2004d. *Dow MOCA Data Management Plan*. March

CH2M HILL. 2004e. *Sample Identification Technical Memorandum.* May

USEPA. 2000. Guidance for the Data Quality Objectives Process (EPA QA/G-4). EPA guidance document EPA/600/R-96/055. August.

Appendix A
Sample Station IDs

Appendix A

Identification of Samples Collected
Sampling and Analysis Plan
Soil Sampling for Bioavailability Study
Dow Midland Off-site Corrective Actions Program

BIOAVAILABILITY LOCATIONS:		SAMPLES COLLECTED FROM EACH BIOAVAILABILITY LOCATION			
Plot Name	**Plot Location**	**Station ID**	**Sample Media**	**Bottom Depth (ft)**	**Sample ID[1]**
Midland 1 East of Plant	Northing 13166306.89 Easting 765447.8698	MNE-02765	Soil[2]	0.1	mmddyy-SOI-02765-00.1
Midland 2 North of Plant	Northing 13160752.26 Easting 767341.0571	MNE-02766	Soil	0.1	mmddyy-SOI-02766-00.1
North of Caldwell Boat Launch	Northing 13168075.82 Easting 754803.287	MIC-02767	Soil	0.5	mmddyy-SOI-02767-00.5
Imerman Park 1	Northing 13198941.53 Easting 713309.9003	THT-02768	Soil	0.5	mmddyy-SOI-02768-00.5
Imerman Park 2	Northing 13198915.07 Easting 712735.9515	THT-02769	Soil	0.5	mmddyy-SOI-02769-00.5
West Michigan Park	Northing 13212823.69 Easting 693205.0275	SHL-02770	Soil	0.5	mmddyy-SOI-02770-00.5

Notes:

1. The "mmddyy" portion of the Sample ID will be replaced in the field with actual date of sample collection.
2. Soil samples will be collected at the surface. Samples will also be collected in accordance with the QAPP.

Appendix B

Site Specific HS&E Plan Amendment

Appendix B

Pilot Study Design: Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils

Pilot Study Design:
Oral Bioavailability of Dioxins/Furans in Midland and Tittabawassee River Flood Plain Soils

The overall objective of this pilot study is to evaluate two animal models (Sprague-Dawley rats and juvenile swine) for measuring the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and the other dioxin/furan congeners of importance in soils from Midland, Michigan, and the Tittabawassee River flood plain. A test soil from each of these two areas will be studied, because the toxic equivalent (TEQ) for dioxins/furans in Midland soils is dominated by TCDD, while that of the Tittabawassee River flood-plain soils is dominated by furans (4-PeCDF in particular). Because the TCDD and 4-PeCDF may behave differently in these two animal models, a soil from each of these two areas will be evaluated in the pilot study. The results from this pilot study will be used to complete the design of a full-scale study of dioxin/furan bioavailability from soil.

Specific objectives of the pilot study include:

- Evaluate the feasibility of detecting dioxins/furans in the tissues of rats and swine dosed with soil from Midland and the Tittabawassee River flood plain
- Evaluate the proposed study design in rats and swine for measuring the relative bioavailability of dioxins/furans in soil
- Establish the absolute oral bioavailability of TCDD and 4-PeCDF from the control doses, so that results from the rat and swine models can be compared directly with each other
- Evaluate whether five animals per dose group will be an adequate number for the full study (note that for the rats in the pilot study, 10 animals will be used and the tissues from each pair of rats will be combined to provide 5 analytical samples).

The study in the rat model will be used to assess the oral bioavailability of dioxins/furans from soil relative to that from both rat feed and oral gavage doses. This is warranted because the cancer slope factor (CSF) for TCDD that was used to calculate a site-specific criterion for dioxins/furans in soil in Midland (Exponent 2002) is based on a study in which rats were dosed with TCDD in feed (see Kociba et al. 1978). Thus, if dioxins/furans in soil are less bioavailable than those in rat feed, an adjustment in the risk assessment is warranted to account for this difference. In addition, the rat studies will allow for comparison to the recent National Toxicology Program (NTP) chronic carcinogenesis bioassays, in which the rats were dosed by gavage.

The swine study will be conducted to evaluate the oral bioavailability of dioxins/furans from two Midland soils in an *in vivo* model that is more similar to humans than the rat, and will provide estimates of both absolute and relative bioavailability (relative to dioxins/furans dosed

in corn oil). The absolute bioavailability estimates in the swine and rats will allow for direct comparison between these two animal models (i.e., the same two soils will be dosed to both models, and estimates of absolute bioavailability from these soils will be obtained in both models).

This document presents the rationale for the pilot study design and discusses the basic study outline, including animal handling, dose preparation and delivery, tissue collection and analysis, data analysis, and reporting. Based on the results from this pilot study, a full-scale study of dioxin/furan bioavailability from soil will be designed, which will include preparation of formal study protocols, consistent with Good Laboratory Practice (GLP) guidelines.

Test Materials

Research has demonstrated that only the fine fraction of soil adheres to human hands and is subject to incidental ingestion. Hand-press trials have indicated that only particles less than approximately 200 μm adhere to the hands of children (Dugan and Inskip 1985). In keeping with this observation, studies of soil ingestion rates in children have found that soil particles in the 0- to 250-μm range are the primary source of ingested soil (Calabrese and Stanek 1996). For this reason, the U.S. Environmental Protection Agency (EPA) has used the <250-μm soil fraction for studies of oral lead bioavailability in humans (Maddaloni et al. 1998), and of lead and arsenic bioavailability in swine (Casteel et al. 1997a,b). Indeed, EPA has stated that "*it is critical to sieve the soil samples to <250 µm (60 mesh) to more closely represent the size of soil particles that would be expected to adhere to children's hands*" (U.S. EPA 1999), when conducting lead bioavailability studies. For these reasons, the <250-μm fraction of the test soils will be used for measurement of dioxin/furan bioavailability, because this is the fraction to which direct-contact exposure would most likely occur.

For the pilot study, two soils will be used—one from Midland and one from the Tittabawassee River flood plain. The Midland soil should have the maximum concentration of TCDD available (approx. 150–200 pg/g)to ensure detection of TCDD in the animal tissues. The Tittabawassee River flood-plain soil, in which the TEQ will be dominated by 4-PeCDF and other furans, should have a TEQ concentration just below 1,000 pg/g (the maximum soil concentration that can be used at the animal testing facility). The test soils will be analyzed for soil parameters (pH, total organic carbon [TOC], and particle size distribution [sand, silt, clay]), and for dioxin/furan content in duplicate, to ensure accurate characterization of the test-soil concentrations used in this study. The test soils will also be analyzed for polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), because the presence of these compounds could confound the results of certain measurements made during the pilot study (discussed below).

Study Design Considerations

Rat Model

The proposed study is designed to determine the relative oral bioavailability of dioxins/furans in soil (i.e., the bioavailability from soil relative to what would have been observed in the critical toxicity study). Because the Kociba et al. (1978) study is the basis for the current CSF for TCDD, the proposed study will employ the same dosing vehicle that was used in the Kociba study as the control dose (Kociba et al. dissolved TCDD in acetone, applied it to rat feed, and dosed the TCDD/rat feed mixture to rats). The relative bioavailability estimate would be directly applicable to human health risk assessment.

However, to compare the results in rats to those in swine, estimates of absolute bioavailability will also be necessary in rats. These data will be obtained by measuring the absolute bioavailability of TCDD and 4-PeCDF from a reference dose, and using this value to correct the relative bioavailability from soil to absolute bioavailability values. Because the distribution of TCDD-like compounds at low doses in the rat depends on the route of administration (Qiao and Riviere 2001), an i.v. dose cannot necessarily be used to establish the absolute bioavailability of an oral dose. Therefore, an oral gavage dose in oil, the absorption of which has been characterized previously in Sprague-Dawley rats (Rose et al. 1976), will be used as the reference dose, on the basis of which the absolute bioavailability from soil will be calculated.

The proposed study will rely on measurement of polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs) in liver and fat after 30 days of repeated dosing; therefore, it is critical to understand the disposition of these compounds to design an appropriate study. In the rat, several CYP-type mixed-function monooxygenase (MFO) enzymes can sequester TCDD and structurally similar compounds, such as PCBs and PAHs, in the liver. Of the MFO enzymes, CYP1A2 appears to bind TCDD most tightly. Therefore, ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-deethylase (MROD) assays will be used to measure CYP1A and CYP1A2 induction in the liver of rats exposed to dioxins/furans. If CYP1A2 is induced to a greater extent in the oral-soil versus oral-control dose groups, then it is reasonable to assume that TCDD sequestration may be occurring in the livers of these animals to a different extent. This would complicate the interpretation of tissue concentration data from the different dose groups. However, if the levels of induction between dose groups are negligible or similar, it can be assumed that TCDD is either not being sequestered, or is being sequestered to a similar extent, in both dose groups. In this case, relative bioavailability can be determined based on relative concentrations in liver tissue between different dose groups.

The minimum dose of TCDD for significant induction of these binding proteins in rats appears to be around 1–10 ng/kg/day (Abraham et al. 1988; Kociba et al. 1978; Leung et al. 1990). The highest concentration of TCDD in Midland soils collected for a previous study of dioxin bioaccessibility was 139 pg TCDD/g soil (Ruby et al. 2002), which would result in a dose of 160 pg TCDD/day (assuming 5% soil in the diet [Sprague-Dawley rats find food unpalatable at greater than 5% soil in feed], and 23 g of feed consumption/day [Freeman et al. 1992]). Because this dose is nearly an order of magnitude below the dose at which enzyme induction becomes important, hepatic sequestration of TCDD is unlikely to occur in the proposed rat

study. However, as discussed above, the activity of the hepatic enzyme CYP1A2 will be measured in the liver of each rat after dosing, to confirm this assumption.

The rats will be dosed with PCDDs/Fs in rat feed for 30 consecutive days to allow body burdens to approach steady state. Measurement of tissue concentrations close to steady-state conditions is less prone to error. The 30-day dosing period was selected as a reasonable length of time based on the observation that the elimination half-life for TCDD body burden in Sprague-Dawley rats averages about 19 days (Geyer et al. 2002). Thus, after 30 days of continuous dosing, TCDD body burdens should be at approximately 65% of steady state, which should be acceptable for conducting the proposed study.

The test soils used for this study must contain a sufficient concentration of PCDDs/Fs to ensure that detectable concentrations of these compounds are present in the rat tissues at the end of the study. The following calculation was performed to determine the minimum concentration of TCDD in the test soils required to ensure detectable tissue levels of TCDD. Assuming that the absolute oral bioavailability of TCDD in soil is 10% (a conservative assumption for the purposes of this calculation), and that the rats will retain 7% of the absorbed dose in their liver (determined using the PBPK model of Leung et al. 1990), a minimum concentration of approximately 10 pg TCDD/g soil would be required for detection of TCDD in liver tissue after 30 days of dosing (assuming 5% soil in feed, 23 g of feed consumption/day, a liver weight of 12 g [Shu et al. 1988], and a method detection limit of 0.2 pg TCDD/g liver tissue). Inclusion of a five-fold margin to ensure accurate quantitation of TCDD would result in a minimum soil concentration of 50 pg TCDD/g soil. However, for the pilot study, the maximum available concentration of TCDD in soil will be used, because the Midland soils contain far lower concentrations of TCDD than have been used in previous *in vivo* studies (Ruby et al. 2002), and it is critical that TCDD be detectable in post-dosing animal tissues for the pilot study to succeed. The Tittabawassee River flood-plain soil will have a TEQ concentration approximately three times that of the Midland soil (approx. 1,000 pg/g), so detection of absorbed furans in the rat tissues should not be a problem.

A study of background concentrations of PCDDs/Fs in the liver and fat of Sprague-Dawley rats due to diet was conducted recently (Ruby et al. 2004) and indicated negligible concentrations of PCDDs/Fs. TCDD concentrations in all samples of liver and fat were below the detection limit (0.0594 pg/g). Concentrations of 4-PeCDF were non-detect (0.0907 pg/g) in the rat fat and were 1.42 pg/g (mean) in the rat livers. Given that dosing a rat with soil containing 50 pg TCDD/g soil for 30 days should result in a liver concentration of approximately 1.0 pg TCDD/g liver (based on the calculation cited above), the background concentrations of TCDD in the rat livers should not pose a problem for this study (i.e., the inter-animal and analytical variability associated with the absorbed dose should be detectable over the background concentrations in the animals). A similar calculation suggests that the concentration of 4-PeCDF detected in rat livers should not pose a problem for this study. However, concentrations of PCDDs/Fs in the rat chow used during the pilot study will be measured to ensure that background concentrations due to diet are not increasing.

Swine Model

The swine study is designed to determine the oral bioavailability of dioxins/furans in soil in a model that bears greater similarity to humans than do rats. The swine data could also be used to adjust the modeled human exposures to PCDDs/Fs in soil that were used to calculate the site-specific criterion for dioxins/furans in soil in Midland, Michigan (Exponent 2002). This would be accomplished by comparing the uptake of dioxins/furans from soil to that from corn oil spiked with the same compounds, to determine the relative bioavailability of the dioxins/furans from soil. The relative bioavailability estimate would be directly applicable to human health risk assessment. This value would then be adjusted for the uptake of TCDD from the corn oil matrix in swine, based on literature values for humans, to obtain an absolute bioavailability value. The absolute bioavailability values for TCDD from the test soil can then be compared to the equivalent value developed in the rat model.

The proposed study will rely on measurement of PCDDs/Fs in liver and fat after 30 consecutive days of dosing. As discussed above, in the rat, the concentration of TCDD that can be attained in the liver is dose dependent and controlled by the induction of one or more hepatic binding proteins. The minimum dose of TCDD in rats that results in detectable, significant induction of these proteins appears to be around 1–10 ng/kg/day (Abraham et al. 1988; Kociba et al. 1978; Leung et al. 1990). Because very little is known about the pharmacokinetics of TCDD in swine, the minimum induction dose in swine was assumed to be similar to that in rats. The highest concentration of TCDD in Midland soils collected for a previous study of dioxin bioaccessibility was 139 pg TCDD/g soil (Ruby et al. 2002), which would result in a dose of 695 pg TCDD/day if a 5-g dose of soil were administered to each of the swine. Because this dose is below the range at which enzyme induction becomes important in rats, significant hepatic sequestration of TCDD is unlikely to occur in the swine study. However, as with the rat study, EROD and MROD activity in swine liver will be measured in all dosing groups to confirm this assumption.

The test soil used for this study must contain a sufficient concentration of dioxins/furans to ensure that detectable concentrations of these compounds are present in the swine tissues at the end of the study. The following calculation was performed to determine the minimum concentration of TCDD in the test soils required to ensure detectable tissue levels of TCDD. Assuming that the absolute oral bioavailability of TCDD in soil is 10% (a conservative assumption for the purposes of this calculation), and that the swine will retain 7% of the absorbed dose in their liver (determined using the PBPK model for rats of Leung et al. [1990], because no such model exists for swine), a minimum concentration of 2 pg TCDD/g soil would be required for detection of TCDD in liver tissue after 30 consecutive days of dosing at 5 g soil/day (assuming analysis of 10 g of liver tissue, and a method detection limit of 0.2 pg TCDD/g liver tissue). Inclusion of a five-fold margin to ensure accurate quantitation of TCDD would result in a minimum soil concentration of 9 pg TCDD/g soil. However, for the pilot study, the maximum available concentration of TCDD in soil will be used, because the Midland soils contain far lower concentrations of TCDD than have been used in previous *in vivo* studies (Ruby et al. 2002), and it is critical that TCDD be detectable in post-dosing animal tissues for the pilot study to succeed. The Tittabawassee River flood-plain soil will have a TEQ concentration approximately three times that of the Midland soil (approx. 1,000 pg/g), so detection of absorbed furans in the swine tissues should not be a problem.

A study of background concentrations of PCDDs/Fs in the liver and fat of juvenile swine due to diet was conducted recently (Ruby et al. 2004) and indicated negligible concentrations of PCDDs/Fs. TCDD and 4-PeCDF concentrations in all samples of liver and fat were below the detection limits (0.0594 pg/g and 0.0907 pg/g, respectively). Thus, the background concentrations of TCDD and 4-PeCDF in the swine livers and fat should not pose a problem for this study (i.e., the inter-animal and analytical variability associated with the absorbed dose should be detectable over the background concentrations in the animals). However, concentrations of PCDDs/Fs in the swine feed used during the pilot study will be measured to ensure that background concentrations due to diet are not increasing.

Test Species Selection and Rationale

Rat Model

Adult, female, Sprague-Dawley rats (4 months of age, approx. 250 g) will be used for this study. This rat model was selected because the dioxin cancer slope factor (CSF) currently in use by the Michigan Department of Environmental Quality (DEQ) was derived from a study in rats (Kociba et al. 1978), and the cancer slope factor presented in EPA's Dioxin Reassessment (U.S. EPA 2000) is based in part on the Kociba rat study. In addition, two previous bioavailability studies of TCDD from soil were conducted in rats (Lucier et al. 1986; Shu et al. 1988). All of the studies cited above used the Sprague-Dawley strain of rat. Female rats will be used, because the CSF in EPA's Dioxin Reassessment (U.S. EPA 2000) is based in part on a benchmark dose assessment of the female rat liver tumor data from Kociba et al. (1978; revised pathology from Goodman and Sauer 1992). All Sprague-Dawley rats will be obtained from Harlan (Indianapolis, Indiana), and maintained on Purina laboratory rodent diet 5001 (the same rodent diet used by Kociba et al. in 1978).

Swine Model

Intact, male juvenile swine (*Sus scrofa*) at 6 weeks of age, and weighing approximately 10 kg, will be used for this study. Swine will be obtained from Chinn Farms (Clarence, Mississippi) and will be fed a specially formulated diet (Ziegler Bros. Inc., Gardners, Pennsylvania) that has been determined to be low in PCDD/F concentrations (Ruby et al. 2004). Juvenile swine were selected as an appropriate surrogate for humans because of the similarity in gastrointestinal physiology between swine and humans. For example, feeding behavior, gastrointestinal anatomy, acid secretion, and the development of small-intestinal absorption mechanisms are all quite similar between swine and humans (Weis and LaVelle 1991). For these reasons, swine have been used as a surrogate for humans in the fields of pharmaceutical research (Dodds 1982) and nutrition (Miller and Ullrey 1987). Juvenile animals were selected, because absorption rates are frequently greater in younger animals, and this model is designed to predict uptake in the most sensitive subpopulation of concern (i.e., children). This test species has been used to assess the oral bioavailability of both lead and arsenic in soil (Casteel et al. 1997a,b), and the results from these studies have been used by EPA to develop relative bioavailability adjustments for human health risk assessments (U.S. EPA 1999; Kelley et al. 2002).

Pilot Study

Rat Study

For the pilot rat study, fifty 4-month-old female Sprague-Dawley rats will be obtained from Harlan and placed in individual stainless steel cages. The rats will be provided Purina laboratory rodent diet 5001 and de-ionized water *ad libitum.* Their health status will be monitored over a one-week quarantine period, and two days prior to dosing, healthy animals will be assigned randomly to test groups.

Ten rats will be used for each dose group, with the tissues from two animals combined to achieve sufficient tissue mass for analysis (i.e., there will be five analyses per dose group). There will be five dose groups in the pilot study: two soil, a feed control, and two oral gavage groups. For the soil dose groups, the two test soils will be blended with the rat chow at 5 wt. % and dosed for 30 days. For the feed control, a blend of dioxins/furans representative of the Midland test soil will be prepared in acetone, blended with rat chow, and dosed for 30 days (see Dose Preparation section below for details). The two oral gavage groups will be dosed with mixtures of dioxins/furans that deliver the same oral doses as the Midland and Tittabawassee soils, but the dioxins/furans will be in corn oil/acetone mixture (99:1; gavage volume of 1 mL); this group will also be dosed for 30 days. Triplicate splits of the soil/chow and feed control/chow mixtures will be tested for TCDD to ensure that homogeneous dosing mixtures have been prepared. Twenty-four hours after the last dose is administered, the animals will be weighed and terminated under anesthesia. Their livers (anticipated to be approx. 10 g) will be excised, blotted dry, and weighed. As much fatty tissue as possible (approx. 4–5 g) will be collected from each rat.

Immediately after sacrifice, the liver samples for the EROD and MROD assays will be collected (1-g samples) from the livers of each pair of rats (i.e., half the sample collected from each liver), snap-frozen, and sent to Michigan State University (MSU) for analysis. The pair of livers will then be frozen and shipped to Alta Analytical, where they will be homogenized together to create a sample of sufficient mass for the planned analyses. As much fatty tissue as possible will be collected from each animal, and combined into a single sample from two rats. The fat samples will be shipped (frozen) to Alta. At Alta, the liver and fat samples will be homogenized, and subsamples will be collected for analysis of lipid content and PCDDs/Fs. In addition, triplicate 25-g subsamples of each blended rodent diet will be collected and shipped to Alta for analysis of dioxins/furans to evaluate the stability of the blended diets during the 30-day dosing period.

The liver and fat samples generated during the pilot study will be analyzed by high-resolution gas chromatography/mass spectrometry (HR-GC/MS; EPA Method 8290) at Alta Analytical Laboratory, Inc. (Alta) in Eldorado Hills, California. Each tissue sample analyzed for dioxins/furans will also be analyzed for lipid content (EPA Method 8290) at Alta, to allow for lipid normalization of the tissue concentration data. Because co-planar PCB concentrations in the liver and fat of Sprague-Dawley rats were uniformly low in the background study (Ruby et al. 2004), only a single liver sample from each dose group will be analyzed for co-planar PCBs

during the pilot study. These samples will be analyzed by HR-GC/MS (EPA Method 1668) by Alta.

The rat livers from the pilot study will be tested to determine whether the CYP1A enzymes have been induced, using EROD and MROD assays, at MSU. If differential induction of CYP1A is observed between dose groups (e.g., oral-soil versus oral-control), then further investigations based on enzyme-specific assays, such as measurement of the protein (western blots) or determination of mRNA for the enzyme, may be applied to elucidate the pattern of MFO induction, and the potential effects on interpretation of the study data.

Rat carcasses from the pilot study will be placed in individual, labeled Ziploc® bags and archived (–80 °C) while the samples are analyzed, and will not be disposed of until the data have been reviewed and it has been determined that no further sampling of the rat carcasses is necessary.

The pilot study in rats will produce the following samples for analysis (Table 1):

- 1 rat-chow sample for PCDDs/Fs
- 18 rat-chow/soil and rat-chow/control homogeneity and stability samples for PCDD/Fs
- 25 liver samples for EROD and MROD assays
- 50 tissue samples (25 each of liver and fat) for lipid content
- 50 tissue samples (25 each liver and fat) for PCDDs/Fs
- 5 liver samples for analysis of co-planar PCBs.

Table 1. Analyses of samples from rats/swine for the pilot bioavailability study

Analysis	Test Soil	Feed	Liver	Fat
PCDDs/Fs (HR-GC/MS)	4	19/1[a]	25/20	25/20
Co-planar PCBs (HR-GC/MS)	2	1/1	5/4	--
Lipid content	--	7/1	25/20	25/20
EROD/MROD assay	--	--	25/20	--

[a] For the rats, a single feed sample will be analyzed for PCDDs/Fs, and triplicate samples of the soil/feed and control dose/feed mixtures will be analyzed to check for homogeneity (TCDD analysis only).

Swine Study

For the pilot swine study, 20 intact, male juvenile swine (*Sus scrofa*) at 6 weeks of age will be obtained from Chinn Farms and fed a specially formulated diet (Ziegler Bros. Inc.) that has been determined to be low in PCDDs/Fs (Ruby et al. 2004). Animals will be housed in stainless-steel pens for a one-week quarantine period prior to dosing. Their health status will be monitored periodically. Two days prior to dosing, healthy animals will be assigned randomly to test groups and placed in individual stainless-steel metabolism cages to acclimate. They will remain in these cages for the duration of the study.

Feeding will occur twice daily, in equal portions, and de-ionized water will be provided *ad libitum*. There will be four dose groups of swine: two soil and two corn oil groups (five swine per dose group). For the soil dose groups, the test soil (10 g/day) will be given as a divided dose using the feed-ball dosing method for 30 consecutive days (see Dose Preparation section below for details). For the corn oil administration groups, dosing will occur by placing the corn oil in gelatin capsules (1 mL/capsule) and embedding each capsule in a feed-ball (see Dose Preparation section below for details). Immediately after dosing, the animals will be given their standard ration of swine feed. Twelve hours after the final dose is administered, the animals will be weighed and terminated under anesthesia.

Immediately after sacrifice, each swine liver will be excised, blotted dry, and weighed. The liver samples for the EROD and MROD assays will be collected (three 1-g samples/liver), snap-frozen, and sent to MSU for analysis. The remainder of the liver will be frozen (–80 °C). The fatty tissue sample will consist of 50–100 g of fat from the abdominal cavity. The liver and fat samples will be shipped to Alta (frozen), where the samples will be homogenized, and subsamples will be collected for analysis of lipid content and PCDDs/Fs. In addition, a 50-g sample of the swine diet will be shipped to Alta for analysis of PCDDs/Fs and co-planar PCBs.

The liver and fat samples generated during the pilot study will be analyzed for PCDDs/Fs by HR-GC/MS (EPA Method 8290) at Alta. Each tissue sample analyzed for dioxins/furans will also be analyzed for lipid content (EPA Method 8290) at Alta, to allow for lipid normalization of the tissue concentration data. Because co-planar PCB concentrations in the liver and fat of juvenile swine were uniformly low in the background study (Ruby et al. 2004), only a single liver sample from each dose group will be analyzed for co-planar PCBs during the pilot study. These samples will be analyzed by HR-GC/MS (EPA Method 1668) at Alta.

The swine livers from the pilot study will be tested to determine whether the CYP1A2 enzyme has been induced, using EROD and MROD assays. If differential induction of CYP1A2 is observed among dose groups (e.g., oral-soil versus oral-control), further investigations based on enzyme-specific assays, such as measurement of the protein (western blots) or determination of mRNA for the enzyme, may be applied to elucidate the pattern of MFO induction and the potential effects on interpretation of the study data.

All swine carcasses from the pilot study will be archived (frozen) while the samples are analyzed, and will not be disposed of until the data have been reviewed and it has been determined that no further sampling of the swine carcasses is necessary.

This pilot study will produce the following samples for analysis (Table 1):

- 1 swine feed sample for analysis of PCDDs/Fs
- 20 liver samples for EROD and MROD assays
- 40 tissue samples (20 each of liver and fat) for lipid content
- 40 tissue samples (20 each of liver and fat) for PCDDs/Fs
- 4 liver samples for analysis of co-planar PCBs.

Dose Preparation and Administration

Rat Study

For the pilot study, test soils containing dioxins/furans (<250-μm size fraction) will be blended with the rat feed (5% w/w). Based on previous studies of this type, female Sprague-Dawley rats will consume approximately 23 g of this mixture per day (Freeman et al. 1992). The rats will be allowed to consume the soil/feed mixture *ad libitum*. The mass consumed by each rat will be recorded every second day (by weighing the remaining feed and calculating the mass consumed by difference), and the feed will be replenished. The mass of any spilled feed will be estimated by the laboratory technician and recorded. These data will be used to calculate the dose received by each rat.

The dosing material for the feed control group will be prepared by dissolving the appropriate concentrations of dioxins/furans in acetone and blending it thoroughly with the rat feed (i.e., the method used by Kociba et al. [1978]). The feed control dosing material will be matched to the Midland test soil, to the extent practicable. This will be accomplished by spiking the five dioxin/furan congeners that contribute the most to the total TEQ in the test soil into acetone, and applying the mixture to rat feed. For example, TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,6,7,8-HpCDD, and 1,2,3,6,7,8-HxCDD account for over 81% of the total TEQ in the Midland soils used in the bioaccessibility study (Ruby et al. 2002). If the study soil shows this set of congeners, then the feed control material matched to that soil will be prepared using these five congeners at the appropriate ratios. The dose of TCDD and the other congeners delivered in the control feed will be prepared so that it is equal to the dose of TCDD delivered in the test soil. The rats will be allowed to consume the control material/feed mixture *ad libitum*. The mass consumed by each rat will be recorded every second day (by weighing the remaining feed and calculating the mass consumed by difference), and the feed will be replenished. The mass of any spilled feed will be estimated by the laboratory technician, and recorded. These data will be used to calculate the dose received by each rat.

The dosing material for the two gavage groups will be prepared by dissolving the appropriate concentrations of dioxins/furans in a corn oil/acetone (99:1) mixture. The gavage dosing materials will be matched to the Midland and Tittabawassee test soils, to the extent practicable. This will be accomplished by spiking the five dioxin/furan congeners that contribute the most to the total TEQ in the test soils, into the corn oil/acetone at the appropriate ratios. The doses of

TCDD, 4-PeCDF, and the other congeners delivered in the gavage doses, will be prepared so that they are equal to the doses delivered in the test soils. A gavage dose of 1 mL of the appropriate corn oil/acetone mixture will be given to each rat in the two gavage dose groups on a daily basis.

Both the soil/feed and control/feed mixtures will be checked for homogeneity prior to dosing by collecting three grab samples and testing these samples for dioxin/furan concentrations. These data will be used to establish doses administered in each of the blended feeds. Subsequent to the 30-day dosing period, triplicate 25-g subsamples of each blended rodent diet will be collected and shipped to Alta for analysis of dioxins/furans to evaluate the stability of the blended diets, and to confirm the doses administered in the blended feeds.

Swine Study

For the swine pilot study, the test-soil doses will be delivered by placing 1 g of the soil in the center of a 20-g moistened dough ball (Zeigler Bros. Swine Diet) and offering it to the swine. The swine will be fasted for two hours prior to dosing, because previous studies conducted in this animal model have indicated that a 2-hour fast will ensure eager acceptance of the 20-g dough ball containing the dose. Five dough balls (containing a total of 5 g of test soil) will be given each morning and afternoon, for a total dose of 10 g soil/day. Immediately after dosing, the animals will be given one-half their standard ration of swine feed. Dosing and feeding will continue twice daily for 30 consecutive days.

The dosing materials for the control groups will be prepared by dissolving the appropriate concentrations of PCDDs/Fs in a corn oil/acetone (99:1) mixture. The corn oil/acetone mixture will be prepared so that 2 mL of this mixture will deliver an equivalent dose to 5 g of the test soil to which it is matched. The corn oil solution will be placed in gel capsules (1 mL/capsule), and these will be embedded in the center of a 20-g ball of moistened swine feed. The feed ball will then be offered to the swine. The control dosing materials will be matched to the test soils, to the extent practicable. This will be accomplished by spiking the five dioxin/furan congeners that contribute the most to the total TEQ in the two test soils into corn oil. For example, TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,6,7,8-HpCDD, and 1,2,3,6,7,8-HxCDD account for over 81% of the total TEQ in the Midland soils used in the bioaccessibility study (Ruby et al. 2002). If the Midland soil shows this congener profile, then the control material matched to this soil will be prepared using these five congeners at the appropriate ratios. The doses of TCDD, 4-PeCDF, and the other congeners delivered in the control doses will be prepared so that they are equal to the doses of these compounds delivered in the test soils. As with the soil dose groups, the control material will be dosed for 30 consecutive days.

Data Analysis

Statistical analyses will be conducted on the data from the pilot study to determine the number of rats and swine needed per dose group in the full study. This will be accomplished by calculating the sample size per group necessary to distinguish the mean soil-dosed tissue concentration from the mean background tissue concentration, and the mean soil-dosed tissue

concentration from the mean control-dosed tissue concentration. Both sample-size calculations will be done using a Type 1 error rate of 0.05 and a power of 0.80 (Type 2 error of 0.20). The number of rats and swine per dose group in the full study will be adjusted based on the larger of these two sample-size determinations. However, if the variance in the pilot study data is such that a reasonable difference cannot be demonstrated with sufficient power, even with a large number of rats or swine per dose group (i.e., >10), then other study parameters (e.g., soil concentration, dosing time, etc.) may have to be changed to increase the power of the study.

The results from the pilot study will also be used to calculate the relative bioavailability of TCDD and 4-PeCDF from the test soils, and associated confidence intervals. This will be accomplished by calculating the mean tissue concentrations of TCDD and 4-PeCDF from the soil and control doses, and the associated standard errors. The uncertainty in the ratio describing relative bioavailability (i.e., mean tissue concentration from soil dose/mean tissue concentration from control dose) will be calculated using a Monte Carlo simulation. The 5^{th} and 95^{th} percentile values from the simulated distribution of relative bioavailability values will be taken as the 90% confidence interval on the relative bioavailability.

Reporting

Once all of the *in vivo* and analytical work has been completed, a study report will be prepared. This report will include a description of the animal handling and dosing procedures, tissue collection, and methods of analysis. Analytical results will be provided in tabular and graphical format, and estimates of the absolute and relative bioavailability of dioxins/furans from the test soil in each of the two animal models will be presented.

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Appendix C

WIL Research Report: Preparation of Diets for a Dietary Exposure Study with Dioxin-Contaminated Soils in Rats

PROJECT TITLE

Preparation of Diets for a Dietary Exposure Study with Dioxin-Contaminated Soils in Rats

PROJECT NUMBER

WIL-518001

CONTRIBUTING SCIENTIST

Daniel W. Sved, Ph.D.
Director, Metabolism and Analytical Chemistry
WIL Research Laboratories, Inc.

PERFORMING LABORATORY

WIL Research Laboratories, Inc.
1407 George Road
Ashland, OH 44805-9281

SPONSOR

Exponent, Inc.
4940 Pearl Circle East
Suite 300
Boulder, CO 80301

GENERAL CONSIDERATIONS

To my knowledge, there were no significant deviations from the intended scope of work or the Standard Operating Procedures of WIL Research Laboratories, Inc. that would be expected to affect the scientific integrity of this study.

_____ _____

Daniel W. Sved, Ph.D. Date
Director, Metabolism and
Analytical Chemistry

PREPARATION OF DIETS FOR A DIETARY EXPOSURE STUDY WITH DIOXIN-CONTAMINATED SOILS IN RATS

1. INTRODUCTION

WIL Research Laboratories, Inc. was subcontracted by Exponent, Inc. to prepare rodent diets containing 5% of Test Soil 1, 5% of Test Soil 2, or a dioxin reference mixture. Samples of the dietary admixes and the basal diet used were sent to Alta Analytical Laboratory for analysis. Dietary admixes and basal diet were shipped to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia.

2. TEST MATERIALS

The following materials were supplied to WIL Research Laboratories for use in preparing the dietary admixes.

A. Test Soil 1

Test Soil 1 was received from Exponent, Inc., Boulder, CO on July 29, 2004 and was assigned WIL Log No. 6256A. The material was labeled with the following information.

CC-S-27 (<250 µm – 2 of 4)
Tag No. 44090

B. Test Soil 2

Test Soil 2 was received from Exponent, Inc., Boulder, CO on July 29, 2004 and was assigned WIL Log No. 6257A. The material was labeled with the following information.

THT02769
Tag No. 57283
(IP2) Test Soil #2
<250 µm

C. Reference Mixture

The reference mixture was received from Alta Analytical Laboratory, El Dorado Hills, CA on August 3, 2004 and was assigned WIL Log No. 6261A. The material was labeled with the following information.

Feed Blending Reference Mixture 040728A
2378-TCDD 0.625 pg/µL

12378-PeCDD 0.3175 pg/µL
123678-HxCDD 0.349 pg/µL
1234678-HpCDD 5.54 pg/µL
23478-PeCDF 0.1715 pg/µL
EXP: 7/28/06

3. BASAL DIET

The basal diet used for this project was PMI International, LLC Certified Rodent LabDiet 5001 (meal). Lot number MAY 17 04 2 was used for the initial dietary admixes prepared on August 4, 2004. Lot number AUG 21 04 3 was used for the additional admix with Test Soil 1 on August 25, 2004; the remaining diet from this lot was shipped to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia.

4. MIXING PROCEDURE

A total batch size of 9.5 kg was prepared for each dietary admix. For the diets containing contaminated soil, 475 g of the appropriate test soil was weighed into a tared vessel. For the diet containing the reference mixture, 100 mL of the reference mixture was measured in a graduated cylinder (to deliver). For each pre-mixture, the test material was transferred to a Hobart mixer containing 1000 g of basal diet and the components mixed for 5 minutes with the speed setting on 1. The pre-mixtures were transferred to a V-blender along with the remaining amount of basal diet needed to achieve the total batch size (8025 g for the soils and 8500 g for the reference mixture). The components were mixed for 15 minutes using the intensifier bar for the first and last 5 minutes. After sample collection (see Section 5), the diet containing the reference mixture remained in an open container for approximately 24 hours to allow the acetone to evaporate.

Based on the analytical results of the dietary admix with Test Soil 1, a second batch of diet containing Test Soil 1 was prepared as previously described. The two dietary admixes with Test Soil 1 were distinguished by their preparation date and were also designated as Mix #1 and Mix #2.

5. SAMPLE COLLECTION AND SHIPMENT

Three samples (25 g each) of each dietary admix were collected into plastic ziplock-type bags. Samples were collected from the initial (bottom), middle, and last (top) portions of the admixes as they were discharged from the V-blender. Samples were shipped under ambient conditions to Alta Analytical Laboratory using an overnight courier. A sample (25 g) of each lot of basal diet used was also sent to Alta Analytical Laboratory.

6. SHIPMENT OF DIETARY ADMIXES

The dietary admixes were shipped under ambient conditions to the Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia using an overnight courier. Each diet was shipped in a separate container. Additionally, any remaining basal diet (lot number AUG 21 04 3) was also shipped.

7. DISPOSITION OF REMAINING TEST MATERIALS

Following shipment of the dietary admixes, all remaining test materials were returned to their respective suppliers.

Appendix D

Detailed Study Data

Table D-1. Rat feed intake during the pilot study

Rat #	Feed Intake (g) Thurs 2-Sep Study Day 2	Sat 4-Sep Study Day 4	Mon 6-Sep Study Day 6	Wed 8-Sep Study Day 8	Fri 10-Sep Study Day 10	Sun 12-Sep Study Day 12	Tues 14-Sep Study Day 14	Thurs 16-Sep Study Day 16	Sat 18-Sep Study Day 18	Mon 20-Sep Study Day 20	Wed 22-Sep Study Day 22	Fri 24-Sep Study Day 24	Sun 26-Sep Study Day 26	Tues 28-Sep Study Day 28	Thurs 30-Sep Study Day 30	Total	Paired Rats	Paired Mean Total Intake (g)
Group 1: Midland Reference Gavage																		
10	30.90	28.15	23.84	25.23	19.61	28.63	25.01	24.04	30.83	23.93	28.18	29.38	23.83	20.04	26.22	387.82	10 & 11	382.68
11	23.14	25.52	21.90	23.56	28.03	23.38	29.31	25.37	27.58	26.16	28.09	25.12	20.92	22.31	27.15	377.54		
12	39.23	35.42	42.91	42.86	44.10	44.71	38.78	38.07	39.28	37.59	44.18	44.95	46.53	47.87	41.38	627.86	12 & 13	489.16
13	34.11	26.53	25.79	27.22	16.28	17.30	19.95	16.80	22.72	19.03	22.88	23.96	22.69	27.97	27.23	350.46		
14	34.72	34.57	33.97	32.77	31.14	31.41	26.76	30.56	24.34	24.16	26.32	35.19	34.13	31.39	28.62	460.05	14 & 15	462.09
15	25.28	28.71	29.40	35.89	26.91	39.28	30.17	36.27	31.27	35.16	29.02	31.53	27.00	32.48	25.76	464.13		
16	21.41	25.78	25.34	21.71	28.05	24.21	28.13	22.22	29.29	23.60	31.88	28.19	29.24	28.60	27.22	394.87	16 & 17	368.95
17	26.05	25.42	23.85	25.74	19.61	23.33	21.49	21.64	27.42	18.17	23.20	21.56	22.15	23.60	19.79	343.02		
18	27.94	23.65	24.69	20.90	20.87	19.23	22.22	23.67	21.62	23.43	22.70	21.04	23.69	22.48	25.46	343.59	18 & 19	372.13
19	26.74	30.29	28.48	29.97	28.04	27.67	26.10	24.55	30.49	25.40	26.14	25.12	22.80	26.24	22.63	400.66		
Gp 1 Mean	**28.95**	**28.40**	**28.02**	**28.59**	**26.26**	**27.92**	**26.79**	**26.32**	**28.48**	**25.66**	**28.26**	**28.60**	**27.30**	**28.30**	**27.15**	**415.00**		
Group 2: Tittabawassee River Flood Plain Soil Reference Gavage																		
20	22.88	13.71	20.15	21.60	24.59	21.08	25.93	30.16	40.76	41.20	36.89	35.53	40.24	38.37	32.08	445.17	20 & 21	399.20
21	27.82	27.28	31.21	28.36	24.04	21.21	19.65	24.75	21.94	21.50	23.94	20.15	20.41	21.67	19.29	353.22		
22	29.60	29.26	30.13	34.56	27.20	22.51	26.48	23.93	27.89	29.63	28.45	27.77	32.20	36.31	32.45	438.37	22 & 23	421.99
23	24.25	24.87	20.96	28.95	30.10	28.13	30.90	24.14	28.44	24.21	23.53	25.74	29.35	26.16	35.87	405.60		
24	21.46	26.91	25.27	26.20	20.23	24.67	24.23	20.61	7.71	18.75						216.04	24 & 29[a]	177.51
25	25.24	22.37	24.36	22.87	25.40	21.85	28.26	20.02	24.08	19.93	22.57	22.55	23.56	40.00	23.60	366.66	25 & 26	384.53
26	30.49	28.22	24.26	26.25	26.60	22.94	26.26	25.24	27.41	25.03	25.33	27.46	25.84	33.39	27.68	402.40		
27	22.47	31.30	26.30	32.00	32.44	26.65	18.02	25.67	14.16	25.98	21.96	20.35	27.59	28.44	28.34	381.67	27 & 28	394.41
28	20.76	27.85	24.19	28.91	22.05	31.53	26.99	27.29	31.86	10.42	27.66	33.36	25.19	35.66	33.42	407.14		
29	25.27	10.58	9.71	19.98	21.89	20.20	22.69	8.66								138.98		
Gp 2 Mean	**25.02**	**24.24**	**23.65**	**26.97**	**25.45**	**24.08**	**24.94**	**23.05**	**24.92**	**24.07**	**26.29**	**26.61**	**28.05**	**32.50**	**29.09**	**400.03[b]**		
Group 3: Midland Soil																		
30	30.56	38.87	38.08	44.21	39.82	41.43	36.49	39.33	42.36	44.57	36.60	40.41	34.77	36.59	34.44	578.53	30 & 31	563.35
31	39.10	37.24	39.90	37.20	38.78	33.99	37.11	36.17	34.54	36.11	31.50	37.80	37.14	33.47	38.11	548.16		
32	36.12	36.55	34.18	35.06	32.80	35.39	35.67	34.40	34.73	30.88	34.07	31.88	33.64	31.64	32.45	509.46	32 & 33	477.20
33	32.25	25.78	33.07	27.88	32.97	31.02	30.42	29.23	28.27	30.80	23.82	33.78	29.62	28.73	27.29	444.93		
34	32.84	35.75	33.95	30.99	35.53	32.66	32.73	36.69	30.90	35.86	29.24	38.06	26.31	32.01	32.95	496.47	34 & 35	504.14
35	26.15	41.41	38.70	33.17	28.10	29.09	40.09	33.26	39.52	34.64	30.24	36.57	29.06	35.90	35.91	511.81		
36	39.49	35.41	29.82	31.03	32.66	34.49	35.37	36.16	33.43	34.56	34.13	30.65	34.55	28.48	34.12	504.35	36 & 37	531.42
37	36.63	39.21	40.25	35.46	38.74	34.92	38.50	41.27	34.23	40.31	32.55	39.04	34.05	34.04	39.29	558.49		
38	34.26	38.86	38.26	34.35	41.77	46.63	42.88	39.59	38.71	40.92	39.53	43.44	37.75	40.87	41.40	599.22	38 & 39	511.01
39	25.21	28.76	28.11	25.51	32.03	24.41	30.35	28.03	27.35	30.73	28.01	30.11	29.03	25.86	29.30	422.80		
Gp 3 Mean	**33.26**	**35.78**	**35.43**	**33.49**	**35.32**	**34.40**	**35.96**	**35.41**	**34.40**	**35.94**	**31.97**	**36.17**	**32.59**	**32.76**	**34.53**	**517.42**		

Table D-1. (cont.)

Rat #	Feed Intake (g) Thurs 2-Sep Study Day 2	Sat 4-Sep Study Day 4	Mon 6-Sep Study Day 6	Wed 8-Sep Study Day 8	Fri 10-Sep Study Day 10	Sun 12-Sep Study Day 12	Tues 14-Sep Study Day 14	Thurs 16-Sep Study Day 16	Sat 18-Sep Study Day 18	Mon 20-Sep Study Day 20	Wed 22-Sep Study Day 22	Fri 24-Sep Study Day 24	Sun 26-Sep Study Day 26	Tues 28-Sep Study Day 28	Thurs 30-Sep Study Day 30	Total	Paired Rats	Paired Mean Total Intake (g)
Group 4: Tittabawassee River Flood Plain Soil																		
40	33.65	38.66	38.54	37.87	37.20	40.83	36.78	39.07	36.72	32.06	36.74	32.79	35.99	34.23	32.17	543.30	40 & 41	548.74
41	37.68	39.97	33.45	33.61	36.88	37.23	40.41	36.59	37.15	40.03	33.15	38.88	33.63	38.95	36.56	554.17		
42	34.72	34.68	33.94	38.78	31.59	34.50	36.89	33.36	37.69	36.41	31.60	40.10	36.73	41.17	41.34	543.50	42 & 43	592.14
43	39.09	35.17	38.22	42.19	39.90	42.54	38.35	43.78	42.75	45.48	44.79	44.82	48.00	47.68	48.02	640.78		
44	37.23	40.66	43.65	36.40	41.92	39.89	38.90	35.37	35.39	34.73	38.78	44.24	43.07	44.75	49.00	603.98	44 & 45	564.56
45	30.89	39.13	34.44	34.12	37.36	33.95	33.26	38.18	34.51	34.46	35.15	35.28	33.99	37.69	32.73	525.14		
46	40.21	41.18	29.44	44.50	45.50	46.00	47.00	48.00	48.00	48.00	48.50	48.75	48.00	48.00	46.00	677.08	46 & 47	605.22
47	34.96	35.32	37.96	35.42	32.30	37.10	37.10	36.86	37.14	34.75	35.46	41.09	29.18	36.85	31.87	533.36		
48	36.75	40.65	31.87	42.85	42.97	43.18	44.73	44.45	42.96	43.00	34.60	39.95	47.50	47.82	40.84	624.12	48 & 49	586.04
49	35.47	37.30	40.20	37.29	38.02	35.71	39.78	37.57	39.46	38.19	34.44	34.59	33.87	34.93	31.14	547.96		
Gp 4 Mean	**36.07**	**38.27**	**36.17**	**38.30**	**38.36**	**39.09**	**39.32**	**39.32**	**39.18**	**38.71**	**37.32**	**40.05**	**39.00**	**41.21**	**38.97**	**579.34**		
Group 5: Midland Reference Feed																		
50	31.91	31.27	30.33	33.24	28.81	28.36	32.27	29.80	30.46	31.79	23.50	35.61	23.50	32.51	29.66	453.02	50 & 51	486.41
51	36.16	41.06	32.11	34.77	34.23	30.11	35.33	30.97	33.72	33.23	30.45	34.85	37.93	38.21	36.66	519.79		
52	28.55	30.22	29.66	28.18	31.03	28.31	27.99	31.11	26.75	29.63	27.68	30.88	28.73	28.47	28.48	435.67	52 & 53	503.40
53	34.50	39.97	37.68	36.08	35.70	37.65	34.52	38.91	40.09	39.56	39.02	43.45	37.95	42.10	33.95	571.13		
54	31.67	34.30	30.25	33.36	26.60	30.32	28.30	31.22	34.17	39.81	39.59	42.06	39.73	29.99	29.85	501.22	54 & 55	481.49
55	29.69	34.22	26.23	30.10	27.07	29.25	28.93	43.18	27.96	31.09	30.39	33.48	26.60	32.13	31.44	461.76		
56	29.63	34.17	32.59	27.61	30.16	24.50	26.73	24.75	29.92	30.44	30.50	30.91	30.41	30.55	32.33	445.20	56 & 57	457.32
57	29.89	33.99	31.46	31.77	36.82	28.83	31.96	30.81	27.27	30.38	28.62	32.45	31.74	29.40	34.05	469.44		
58	34.65	35.41	33.90	33.40	31.98	18.27	25.06	22.12	27.21	20.79	30.70	29.42	26.57	35.66	25.55	430.69	58 & 59	446.53
59	31.01	38.09	27.75	32.29	29.45	31.78	28.00	33.90	28.05	30.72	30.90	34.32	26.06	33.73	26.32	462.37		
Gp 5 Mean	**31.77**	**35.27**	**31.20**	**32.08**	**31.19**	**28.74**	**29.91**	**31.68**	**30.56**	**31.74**	**31.14**	**34.74**	**30.92**	**33.28**	**30.83**	**475.03**		

Note: Rats were offered 50 g of feed every 2 days.

[a] Rats #29 and #24 were sacrificed after 15 and 20 days of dosing, respectively, due to persistent problems with administering the gavage dose.

[b] Mean excludes the rat-pair who were sacrificed early.

Table D-2. Rat body weights during the pilot study

Rat #	Body Weight (g) Wed 25-Aug Study Day -6	Sun 29-Aug Study Day -2	Fri 3-Sep Study Day 3	Fri 10-Sep Study Day 10	Fri 17-Sep Study Day 17	Fri 24-Sep Study Day 24	Thurs 30-Sep Study Day 30[a]	Paired Rats	Body Weight (g) Mean Rat Pair Day -2 to 30	Terminal Rat Pair Study Day 30
Group 1: Midland Reference Gavage										
10	227.32	246.74	258.92	251.80	266.22	282.71	269.57	10 & 11	252.37	256.46
11	229.82	238.80	238.90	240.84	242.45	248.09	243.34			
12	226.01	242.66	245.58	259.82	258.01	277.81	288.96	12 & 13	262.33	281.82
13	229.22	258.70	259.02	259.66	257.20	265.83	274.68			
14	219.83	236.64	240.49	243.53	241.81	252.26	254.91	14 & 15	245.17	250.93
15	228.14	235.91	240.85	241.91	252.76	253.98	246.94			
16	228.16	243.27	240.50	244.33	241.37	254.86	257.21	16 & 17	246.37	254.55
17	218.67	233.56	239.90	244.61	249.99	254.95	251.89			
18	228.96	239.78	238.06	239.44	244.73	249.92	249.19	18 & 19	247.63	249.86
19	230.51	240.52	247.95	257.30	256.59	257.55	250.53			
Grp 1 Mean	**226.66**	**241.66**	**245.02**	**248.32**	**251.11**	**259.80**	**258.72**	**Grp 1 Mean**		**258.72**
Group 2: Tittabawassee River Flood Plain Soil Reference Gavage										
20	220.95	227.82	228.82	235.86	237.27	242.49	241.92	20 & 21	241.04	247.42
21	215.93	229.15	238.31	250.70	250.24	256.93	252.91			
22	216.30	232.21	240.34	245.98	248.71	257.53	254.62	22 & 23	244.28	252.49
23	222.97	233.76	234.67	238.12	242.19	252.85	250.35			
24	219.39	238.29	238.52	245.57	232.51	dead	dead	24 & 29[b]	241.20	235.56
25	223.76	236.98	241.36	252.20	253.43	263.57	247.84	25 & 26	251.07	253.67
26	220.20	240.19	245.25	251.37	258.07	263.12	259.50			
27	226.74	234.89	244.43	266.72	248.68	263.82	267.74	27 & 28	244.09	251.25
28	225.60	232.88	236.09	226.65	233.36	239.07	234.75			
29	232.55	251.32	241.08	238.61	dead	dead	dead			
Grp 2 Mean	**222.44**	**235.75**	**238.89**	**245.18**	**244.94**	**254.92**	**251.20**	**Grp 2 Mean[c]**	**245.12**	**251.20**
Group 3: Midland Soil										
30	224.12	234.36	238.46	235.65	243.17	251.54	247.03	30 & 31	256.46	271.69
31	226.45	249.07	256.49	268.64	275.24	281.48	296.35			
32	223.26	239.38	240.67	249.19	256.64	260.05	269.55	32 & 33	244.42	257.19
33	216.93	228.26	227.48	233.10	237.93	245.91	244.83			
34	229.36	244.44	252.32	260.91	265.09	273.68	275.34	34 & 35	259.73	269.76
35	235.12	255.84	252.18	250.47	260.22	262.11	264.18			
36	226.34	246.22	256.72	247.32	249.44	253.71	253.92	36 & 37	246.55	252.98
37	218.35	231.69	234.07	240.16	241.81	251.51	252.03			
38	217.60	240.04	247.90	254.77	262.18	269.07	267.45	38 & 39	248.39	258.44
39	217.80	229.49	232.51	239.83	238.69	249.30	249.42			
Grp 3 Mean	**223.53**	**239.88**	**243.88**	**248.00**	**253.04**	**259.84**	**262.01**	**Grp 3 Mean**		**262.01**
Group 4: Tittabawassee River Flood Plain Soil										
40	220.50	230.42	242.09	252.31	256.30	255.30	259.90	40 & 41	251.24	259.24
41	229.74	244.26	251.25	249.37	252.92	262.17	258.57			
42	220.20	237.93	244.79	248.40	255.40	269.29	280.21	42 & 43	251.77	274.75
43	210.02	230.84	237.51	241.39	250.97	255.27	269.29			
44	237.98	247.10	258.64	270.70	270.68	277.48	281.41	44 & 45	263.97	277.66
45	219.99	242.52	247.50	259.93	263.70	274.05	273.90			
46	217.86	236.47	242.25	244.96	256.07	256.43	251.95	46 & 47	250.95	258.63
47	219.88	234.70	241.04	247.78	259.45	274.94	265.31			
48	218.96	236.69	240.94	242.03	245.77	251.14	253.08	48 & 49	241.30	248.76
49	213.48	227.81	230.45	235.76	239.15	248.33	244.44			
Grp 4 Mean	**220.86**	**236.87**	**243.65**	**249.26**	**255.04**	**262.44**	**263.81**	**Grp 4 Mean**		**263.81**

Table D-2. (cont.)

Rat #	Body Weight (g) Wed 25-Aug Study Day -6	Sun 29-Aug Study Day -2	Fri 3-Sep Study Day 3	Fri 10-Sep Study Day 10	Fri 17-Sep Study Day 17	Fri 24-Sep Study Day 24	Thurs 30-Sep Study Day 30[a]	Paired Rats	Body Weight (g) Mean Rat Pair Day -2 to 30	Terminal Rat Pair Study Day 30
Group 5: Midland Reference Feed										
50	221.21	234.91	240.96	247.44	247.89	255.09	250.36	50 & 51	254.29	268.32
51	226.42	243.77	253.09	259.16	262.19	270.40	286.27			
52	216.44	226.50	231.82	241.35	245.22	254.67	253.28	52 & 53	238.93	250.18
53	217.16	226.77	224.07	237.05	239.51	239.90	247.07			
54	226.09	234.14	240.47	247.37	254.51	268.93	259.72	54 & 55	252.63	257.96
55	236.74	248.40	250.35	254.48	257.48	259.46	256.20			
56	218.55	231.66	237.84	236.25	237.59	246.56	250.95	56 & 57	247.91	259.44
57	220.33	240.30	244.43	251.31	253.39	276.68	267.92			
58	249.56	247.41	258.25	267.36	262.41	263.04	260.69	58 & 59	253.43	255.36
59	223.14	238.72	241.52	245.88	248.78	257.05	250.03			
Grp 5 Mean	**225.56**	**237.26**	**242.28**	**248.77**	**250.90**	**259.18**	**258.25**	**Grp 5 Mean**		**258.25**

[a] Weight after death.

[b] Rats #29 and #24 were sacrificed after 15 and 20 days of dosing, respectively, due to persistent problems with administering the gavage dose

[c] Mean excludes the rat-pair who were sacrificed early.

Table D-3. Rat necropsy liver and fat sample weights

Rat #	Liver Weight (g)	Abdominal Fat Sample Weight (g)	Paired Rats	Liver Weight Average (by pair) (g)	Abdominal Fat Sample Weight (by pair, sum) (g)
Group 1: Midland Reference Gavage					
10	10.26	3.84	10 & 11	8.95	8.33
11	7.63	4.49			
12	9.87	4.32	12 & 13	9.60	7.44
13	9.33	3.12			
14	9.45	4.46	14 & 15	9.00	8.32
15	8.54	3.86			
16	8.09	3.76	16 & 17	8.35	8.31
17	8.60	4.55			
18	8.55	4.12	18 & 19	8.31	9.09
19	8.07	4.97			
Gp 1 Mean	**8.84**	**4.15**			
Group 2: Tittabawassee River Flood Plain Soil Reference Gavage					
20	8.09	4.09	20 & 21	8.44	8.66
21	8.78	4.57			
22	9.23	4.93	22 & 23	8.67	10.76
23	8.11	5.83			
24	9.44 [a]	1.02 [a]	24 & 29	8.97	3.88
25	7.33	3.06	25 & 26	8.26	7.27
26	9.18	4.21			
27	9.18	6.67	27 & 28	8.54	10.94
28	7.89	4.27			
29	8.50 [b]	2.86 [b]			
Gp 2 Mean	**8.57**	**4.15**			
Group 3: Midland Soil					
30	7.95	3.04	30 & 31	9.68	9.61
31	11.40	6.57			
32	9.08	4.94	32 & 33	8.50	8.35
33	7.91	3.41			
34	9.63	4.96	34 & 35	9.68	9.63
35	9.73	4.67			
36	9.08	3.92	36 & 37	9.21	7.37
37	9.34	3.45			
38	9.73	4.56	38 & 39	9.18	8.56
39	8.63	4.00			
Gp 3 Mean	**9.25**	**4.35**			
Group 4: Tittabawassee River Flood Plain Soil					
40	9.31	4.77	40 & 41	9.11	8.21
41	8.91	3.44			
42	11.13	4.87	42 & 43	10.76	10.33
43	10.39	5.46			
44	9.90	4.57	44 & 45	9.79	7.26
45	9.68	2.69			
46	7.51	4.04	46 & 47	8.31	7.96
47	9.10	3.92			
48	8.59	3.41	48 & 49	8.49	6.69
49	8.38	3.28			
Gp 4 Mean	**9.29**	**4.05**			

Table D-3. (cont.)

Rat #	Liver Weight (g)	Abdominal Fat Sample Weight (g)	Paired Rats	Liver Weight Average (by pair) (g)	Abdominal Fat Sample Weight (by pair, sum) (g)
Group 5: Midland Reference Feed					
50	9.26	3.41	50 & 51	9.64	8.94
51	10.02	5.53			
52	8.62	4.01	52 & 53	8.39	8.96
53	8.16	4.95			
54	9.69	4.40	54 & 55	9.29	7.66
55	8.88	3.26			
56	9.52	3.81	56 & 57	9.70	8.10
57	9.87	4.29			
58	9.44	3.89	58 & 59	9.25	8.29
59	9.05	4.40			
Gp 5 Mean	**9.25**	**4.20**			

Notes:

Liver was weighed, EROD/MROD sample cut out, remainder wrapped in foil and placed on dry ice.
For fat samples, samplers tried to get 4–5 g from same areas on all rats. Fat samples were weighed, wrapped in foil, and placed on dry ice

[a] Sample was taken on 9/20/04 before study termination.
[b] Sample was taken on 9/16/04 before study termination.

Table D-4. Rat liver microsomal EROD and MROD activities

Group	Entrix Sample ID	Exponent ID	EROD (pmol/mg/min)	MROD (pmol/mg/min)
1	ERL-1	10 & 11	257.5	120.6
1	ERL-2	12 & 13	168.4	111.9
1	ERL-3	14 & 15	115.8	95.4
1	ERL-4	16 & 17	151.2	104.9
1	ERL-5	18 & 19	153.1	108.6
2	ERL-6	20 & 21	486.1	196.5
2	ERL-7	22 & 23	430.0	176.2
2	ERL-26	24[a]	489.4	101.1
2	ERL-8	25 & 26	406.6	68.6
2	ERL-9	27 & 28	455.3	209.1
3	ERL-10	30 & 31	99.1	93.0
3	ERL-11	32 & 33	75.7	95.3
3	ERL-12	34 & 35	84.4	119.6
3	ERL-13	36 & 37	91.4	115.6
3	ERL-15	38 & 39	62.5	80.9
4	ERL-16	40 & 41	261.1	148.3
4	ERL-17	42 & 43	319.0	139.3
4	ERL-18	44 & 45	307.2	198.3
4	ERL-19	46 & 47	346.8	154.3
4	ERL-20	48 & 49	361.5	198.0
5	ERL-21	50 & 51	152.5	120.0
5	ERL-22	52 & 53	151.9	139.1
5	ERL-23	54 & 55	128.3	117.7
5	ERL-24	56 & 57	146.7	136.8
5	ERL-25	58 & 59	120.9	96.2

Note: All assays conducted as outlined in SOP250 MSU-ATL SOP 250 version 1
Sample #29 was not analyzed due to ampule breakage and loss of sample in transit.

[a] Results excluded from analyses because this animal was sacrificed before end of study.

Table D-5. Tissue concentrations, doses, and RBA calculations for the rat pilot study: Midland soil

	Midland Soil (Group 3)											
	Soil CC-S-27/ Diet Blend (Test Article #1)						Using Mean BW					
Analyte	Mean Conc. (pg/g)	% of TEQ (in soil)	Group 3 Rat IDs	Total Feed Intake (g)	Mean BW (g)	Terminal BW (g)	Total Dose (pg/g BW)	Avg. Daily Dose (pg/g BW/d)	Avg. Daily Dose S.D.	Total Dose (pg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)
2,3,7,8-TCDD	4.40	48.9%	Grp 3 Mean	517.42	251.11	262.01	9.07	0.302	0.017	2,277		
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	Grp 3 Mean	517.42	251.11	262.01	5.15	0.172	0.010	1,294		
1,2,3,6,7,8-HxCDD	3.59	2.7%	Grp 3 Mean	517.42	251.11	262.01	7.40	0.247	0.014	1,858		
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	Grp 3 Mean	517.42	251.11	262.01	145	4.822	0.271	36,323		
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	Grp 3 Mean	517.42	251.11	262.01	2.99	0.100	0.006	750		
2,3,7,8-TCDD	4.40	48.9%	30 & 31	563.35	256.46	271.69	9.67	0.322		2,479	9.68	9.81
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	30 & 31	563.35	256.46	271.69	5.49	0.183		1,408	9.68	12.6
1,2,3,6,7,8-HxCDD	3.59	2.7%	30 & 31	563.35	256.46	271.69	7.89	0.263		2,022	9.68	32.0
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	30 & 31	563.35	256.46	271.69	154	5.140		39,547	9.68	335
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	30 & 31	563.35	256.46	271.69	3.19	0.106		817	9.68	21.1
2,3,7,8-TCDD	4.40	48.9%	32 & 33	477.20	244.42	257.19	8.59	0.286		2,100	8.50	11.3
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	32 & 33	477.20	244.42	257.19	4.88	0.163		1,193	8.50	14.0
1,2,3,6,7,8-HxCDD	3.59	2.7%	32 & 33	477.20	244.42	257.19	7.01	0.234		1,713	8.50	37.3
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	32 & 33	477.20	244.42	257.19	137	4.569		33,499	8.50	387
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	32 & 33	477.20	244.42	257.19	2.83	0.094		692	8.50	24.1
2,3,7,8-TCDD	4.40	48.9%	34 & 35	504.14	259.73	269.76	8.54	0.285		2,218	9.68	9.35
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	34 & 35	504.14	259.73	269.76	4.85	0.162		1,260	9.68	11.1
1,2,3,6,7,8-HxCDD	3.59	2.7%	34 & 35	504.14	259.73	269.76	6.97	0.232		1,810	9.68	29.7
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	34 & 35	504.14	259.73	269.76	136	4.542		35,391	9.68	318
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	34 & 35	504.14	259.73	269.76	2.81	0.094		731	9.68	20.1
2,3,7,8-TCDD	4.40	48.9%	36 & 37	531.42	246.55	252.98	9.48	0.316		2,338	9.21	10.8
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	36 & 37	531.42	246.55	252.98	5.39	0.180		1,329	9.21	13.7
1,2,3,6,7,8-HxCDD	3.59	2.7%	36 & 37	531.42	246.55	252.98	7.74	0.258		1,908	9.21	34.2
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	36 & 37	531.42	246.55	252.98	151	5.044		37,306	9.21	363
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	36 & 37	531.42	246.55	252.98	3.13	0.104		771	9.21	22.8
2,3,7,8-TCDD	4.40	48.9%	38 & 39	511.01	248.39	258.44	9.05	0.302		2,248	9.18	10.7
1,2,3,7,8-PeCDD	2.50 *J*	24.9%	38 & 39	511.01	248.39	258.44	5.14	0.171		1,278	9.18	13.4
1,2,3,6,7,8-HxCDD	3.59	2.7%	38 & 39	511.01	248.39	258.44	7.39	0.246		1,835	9.18	33.3
1,2,3,4,6,7,8-HpCDD	70.2	4.3%	38 & 39	511.01	248.39	258.44	144	4.814		35,873	9.18	347
2,3,4,7,8-PeCDF	1.45 *J*	6.7%	38 & 39	511.01	248.39	258.44	2.98	0.099		741	9.18	22.7

Table D-5. (cont.)

						Midland Soil (Group 3)						
			Using Terminal BW									
Analyte	WHO TEF (unitless)	Liver TEQ (pg/g)	Fat Weight Fraction (w_a) (unitless)	Fat Weight (g)	Fat Conc. (pg/g)	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.	RBA Grp 3: Grp 1 Indiv: Grp Mean Using FR_{sum} (unitless)
2,3,7,8-TCDD	1					0.042	0.003	0.120	0.016	0.162	0.017	35%
1,2,3,7,8-PeCDD	1					0.093	0.006	0.113	0.016	0.206	0.016	40%
1,2,3,6,7,8-HxCDD	0.1					0.166	0.012	0.065	0.008	0.230	0.016	47%
1,2,3,4,6,7,8-HpCDD	0.01					0.089	0.006	0.015	0.002	0.104	0.007	34%
2,3,4,7,8-PeCDF	0.5					0.273	0.017	0.042	0.006	0.315	0.018	40%
2,3,7,8-TCDD	1	9.81	0.0707	19.21	12.7	0.038		0.098		0.137		0.298
1,2,3,7,8-PeCDD	1	12.6	0.0707	19.21	6.91 *J*	0.087		0.094		0.181		0.351
1,2,3,6,7,8-HxCDD	0.1	3.2	0.0707	19.21	5.83 *J*	0.153		0.055		0.209		0.423
1,2,3,4,6,7,8-HpCDD	0.01	3.35	0.0707	19.21	25.5	0.082		0.012		0.094		0.308
2,3,4,7,8-PeCDF	0.5	10.55	0.0707	19.21	1.57 *J*	0.250		0.037		0.287		0.360
2,3,7,8-TCDD	1	11.3	0.0678	17.44	14.4	0.046		0.120		0.165		0.361
1,2,3,7,8-PeCDD	1	14	0.0678	17.44	8.00 *J*	0.100		0.117		0.217		0.421
1,2,3,6,7,8-HxCDD	0.1	3.73	0.0678	17.44	6.67 *J*	0.185		0.068		0.253		0.513
1,2,3,4,6,7,8-HpCDD	0.01	3.87	0.0678	17.44	29.3	0.098		0.015		0.113		0.371
2,3,4,7,8-PeCDF	0.5	12.05	0.0678	17.44	1.63 *J*	0.296		0.041		0.337		0.423
2,3,7,8-TCDD	1	9.35	0.0703	18.97	16.9	0.041		0.145		0.185		0.404
1,2,3,7,8-PeCDD	1	11.1	0.0703	18.97	9.16 *J*	0.085		0.138		0.223		0.433
1,2,3,6,7,8-HxCDD	0.1	2.97	0.0703	18.97	7.22 *J*	0.159		0.076		0.235		0.475
1,2,3,4,6,7,8-HpCDD	0.01	3.18	0.0703	18.97	33.3	0.087		0.018		0.105		0.342
2,3,4,7,8-PeCDF	0.5	10.05	0.0703	18.97	2.01 *J*	0.266		0.052		0.318		0.400
2,3,7,8-TCDD	1	10.8	0.0670	16.94	16.1	0.043		0.117		0.159		0.347
1,2,3,7,8-PeCDD	1	13.7	0.0670	16.94	8.52 *J*	0.095		0.109		0.204		0.395
1,2,3,6,7,8-HxCDD	0.1	3.42	0.0670	16.94	7.34 *J*	0.165		0.065		0.230		0.467
1,2,3,4,6,7,8-HpCDD	0.01	3.63	0.0670	16.94	32.9	0.090		0.015		0.105		0.341
2,3,4,7,8-PeCDF	0.5	11.4	0.0670	16.94	1.77 *J*	0.273		0.039		0.311		0.391
2,3,7,8-TCDD	1	10.7	0.0681	17.59	15.3	0.044		0.120		0.163		0.356
1,2,3,7,8-PeCDD	1	13.4	0.0681	17.59	7.88 *J*	0.096		0.109		0.205		0.397
1,2,3,6,7,8-HxCDD	0.1	3.33	0.0681	17.59	6.10 *J*	0.167		0.058		0.225		0.456
1,2,3,4,6,7,8-HpCDD	0.01	3.47	0.0681	17.59	29.9	0.089		0.015		0.103		0.338
2,3,4,7,8-PeCDF	0.5	11.35	0.0681	17.59	1.62 *J*	0.281		0.038		0.320		0.401

Table D-5. (cont.)

	Midland Reference Feed (Group 5)										
	Acetone Mixture/ Feed Blend (Test Article #3)		Total			Using Mean BW				Liver	
	Mean Conc.	Group 5	Feed Intake	Mean BW	Terminal BW	Total Dose	Avg. Daily Dose	Avg. Daily Dose	Total Dose	Weight (mean)	Liver Conc.
Analyte	(pg/g)	Rat IDs	(g)	(g)	(g)	(pg/g BW)	(pg/g BW/d)	S.D.	(pg)	(g)	(pg/g)
2,3,7,8-TCDD	5.54	Grp 5 Mean	475.03	249.44	258.25	10.6	0.352	0.024	2,632		
1,2,3,7,8-PeCDD	3.50	Grp 5 Mean	475.03	249.44	258.25	6.67	0.222	0.015	1,663		
1,2,3,6,7,8-HxCDD	4.49	Grp 5 Mean	475.03	249.44	258.25	8.55	0.285	0.019	2,133		
1,2,3,4,6,7,8-HpCDD	55.6	Grp 5 Mean	475.03	249.44	258.25	106	3.533	0.236	26,412		
2,3,4,7,8-PeCDF	1.91 *J*	Grp 5 Mean	475.03	249.44	258.25	3.64	0.121	0.008	907		
2,3,7,8-TCDD	5.54	50 & 51	486.41	254.29	268.32	10.6	0.353		2,695	9.64	30.8
1,2,3,7,8-PeCDD	3.50	50 & 51	486.41	254.29	268.32	6.69	0.223		1,702	9.64	33.7
1,2,3,6,7,8-HxCDD	4.49	50 & 51	486.41	254.29	268.32	8.59	0.286		2,184	9.64	62.4
1,2,3,4,6,7,8-HpCDD	55.6	50 & 51	486.41	254.29	268.32	106	3.545		27,044	9.64	440
2,3,4,7,8-PeCDF	1.91 *J*	50 & 51	486.41	254.29	268.32	3.65	0.122		929	9.64	52.6
2,3,7,8-TCDD	5.54	52 & 53	503.40	238.93	250.18	11.7	0.389		2,789	8.39	29.7
1,2,3,7,8-PeCDD	3.50	52 & 53	503.40	238.93	250.18	7.37	0.246		1,762	8.39	33.6
1,2,3,6,7,8-HxCDD	4.49	52 & 53	503.40	238.93	250.18	9.46	0.315		2,260	8.39	65.6
1,2,3,4,6,7,8-HpCDD	55.6	52 & 53	503.40	238.93	250.18	117	3.905		27,989	8.39	467
2,3,4,7,8-PeCDF	1.91 *J*	52 & 53	503.40	238.93	250.18	4.02	0.134		961	8.39	56.1
2,3,7,8-TCDD	5.54	54 & 55	481.49	252.63	257.96	10.6	0.352		2,667	9.29	32.8
1,2,3,7,8-PeCDD	3.50	54 & 55	481.49	252.63	257.96	6.67	0.222		1,685	9.29	36.2
1,2,3,6,7,8-HxCDD	4.49	54 & 55	481.49	252.63	257.96	8.56	0.285		2,162	9.29	68.1
1,2,3,4,6,7,8-HpCDD	55.6	54 & 55	481.49	252.63	257.96	106	3.532		26,771	9.29	470
2,3,4,7,8-PeCDF	1.91 *J*	54 & 55	481.49	252.63	257.96	3.64	0.121		920	9.29	55.8
2,3,7,8-TCDD	5.54	56 & 57	457.32	247.91	259.44	10.2	0.341		2,534	9.70	31.0
1,2,3,7,8-PeCDD	3.50	56 & 57	457.32	247.91	259.44	6.46	0.215		1,601	9.70	33.9
1,2,3,6,7,8-HxCDD	4.49	56 & 57	457.32	247.91	259.44	8.28	0.276		2,053	9.70	63.2
1,2,3,4,6,7,8-HpCDD	55.6	56 & 57	457.32	247.91	259.44	103	3.419		25,427	9.70	449
2,3,4,7,8-PeCDF	1.91 *J*	56 & 57	457.32	247.91	259.44	3.52	0.117		873	9.70	55.1
2,3,7,8-TCDD	5.54	58 & 59	446.53	253.43	255.36	9.8	0.325		2,474	9.25	32.0
1,2,3,7,8-PeCDD	3.50	58 & 59	446.53	253.43	255.36	6.17	0.206		1,563	9.25	33.7
1,2,3,6,7,8-HxCDD	4.49	58 & 59	446.53	253.43	255.36	7.91	0.264		2,005	9.25	61.3
1,2,3,4,6,7,8-HpCDD	55.6	58 & 59	446.53	253.43	255.36	98	3.265		24,827	9.25	437
2,3,4,7,8-PeCDF	1.91 *J*	58 & 59	446.53	253.43	255.36	3.37	0.112		853	9.25	54.2

Table D-5. (cont.)

Analyte	WHO TEF (unitless)	Liver TEQ (pg/g)	Midland Reference Feed (Group 5) Using Terminal BW Fat Weight Fraction (w_a) (unitless)	Fat Weight (g)	Fat Conc. (pg/g)	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.
2,3,7,8-TCDD	1					0.110	0.012	0.263	0.030	0.373	0.042
1,2,3,7,8-PeCDD	1					0.191	0.018	0.182	0.022	0.373	0.039
1,2,3,6,7,8-HxCDD	0.1					0.279	0.022	0.080	0.014	0.359	0.033
1,2,3,4,6,7,8-HpCDD	0.01					0.159	0.012	0.021	0.003	0.180	0.014
2,3,4,7,8-PeCDF	0.5					0.560	0.046	0.063	0.006	0.623	0.051
2,3,7,8-TCDD	1	30.8	0.0700	18.79	38.9	0.110		0.271		0.381	
1,2,3,7,8-PeCDD	1	33.7	0.0700	18.79	17.4	0.191		0.192		0.383	
1,2,3,6,7,8-HxCDD	0.1	6.24	0.0700	18.79	9.96 *J*	0.275		0.086		0.361	
1,2,3,4,6,7,8-HpCDD	0.01	4.4	0.0700	18.79	32.7	0.157		0.023		0.180	
2,3,4,7,8-PeCDF	0.5	26.3	0.0700	18.79	3.13 *J*	0.546		0.063		0.609	
2,3,7,8-TCDD	1	29.7	0.0664	16.62	35.6	0.089		0.212		0.301	
1,2,3,7,8-PeCDD	1	33.6	0.0664	16.62	15.4	0.160		0.145		0.305	
1,2,3,6,7,8-HxCDD	0.1	6.56	0.0664	16.62	7.95 *J*	0.244		0.058		0.302	
1,2,3,4,6,7,8-HpCDD	0.01	4.67	0.0664	16.62	27.1	0.140		0.016		0.156	
2,3,4,7,8-PeCDF	0.5	28.05	0.0664	16.62	3.22 *J*	0.490		0.056		0.545	
2,3,7,8-TCDD	1	32.8	0.0680	17.53	41.9	0.114		0.275		0.390	
1,2,3,7,8-PeCDD	1	36.2	0.0680	17.53	17.3	0.200		0.180		0.380	
1,2,3,6,7,8-HxCDD	0.1	6.81	0.0680	17.53	9.95 *J*	0.293		0.081		0.373	
1,2,3,4,6,7,8-HpCDD	0.01	4.7	0.0680	17.53	32.9	0.163		0.022		0.185	
2,3,4,7,8-PeCDF	0.5	27.9	0.0680	17.53	3.23 *J*	0.564		0.062		0.625	
2,3,7,8-TCDD	1	31	0.0683	17.71	37.5	0.119		0.262		0.381	
1,2,3,7,8-PeCDD	1	33.9	0.0683	17.71	17.1	0.205		0.189		0.395	
1,2,3,6,7,8-HxCDD	0.1	6.32	0.0683	17.71	9.09 *J*	0.299		0.078		0.377	
1,2,3,4,6,7,8-HpCDD	0.01	4.49	0.0683	17.71	29.4	0.171		0.020		0.192	
2,3,4,7,8-PeCDF	0.5	27.55	0.0683	17.71	3.13 *J*	0.612		0.063		0.675	
2,3,7,8-TCDD	1	32	0.0675	17.23	42.1	0.120		0.293		0.413	
1,2,3,7,8-PeCDD	1	33.7	0.0675	17.23	18.5	0.199		0.204		0.403	
1,2,3,6,7,8-HxCDD	0.1	6.13	0.0675	17.23	11.4	0.283		0.098		0.381	
1,2,3,4,6,7,8-HpCDD	0.01	4.37	0.0675	17.23	34.2	0.163		0.024		0.187	
2,3,4,7,8-PeCDF	0.5	27.1	0.0675	17.23	3.63 *J*	0.588		0.073		0.661	

Table D-5. (cont.)

	Midland Reference Gavage (Group 1)										
	Reference Mixture #1					Using Mean BW					
Analyte	Mean Conc. (ng/mL)	Group 1 Rat IDs	Total Gavage Volume (mL)	Mean BW (g)	Terminal BW (g)	Total Dose (pg/g BW)	Avg. Daily Dose (pg/g BW)	Avg. Daily Dose S.D.	Total Dose (pg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)
2,3,7,8-TCDD	0.128	Grp 1 Mean	30	250.77	258.72	15.3	0.511	0.014	3,840		
1,2,3,7,8-PeCDD	0.0740	Grp 1 Mean	30	250.77	258.72	8.85	0.295	0.008	2,220		
1,2,3,6,7,8-HxCDD	0.106	Grp 1 Mean	30	250.77	258.72	12.7	0.423	0.012	3,180		
1,2,3,4,6,7,8-HpCDD	1.33	Grp 1 Mean	30	250.77	258.72	159	5.307	0.145	39,900		
2,3,4,7,8-PeCDF	0.0397	Grp 1 Mean	30	250.77	258.72	4.75	0.158	0.004	1,191		
2,3,7,8-TCDD	0.128	10 & 11	30	252.37	256.46	15.2	0.507		3,840	8.95	59.7
1,2,3,7,8-PeCDD	0.0740	10 & 11	30	252.37	256.46	8.80	0.293		2,220	8.95	63.4
1,2,3,6,7,8-HxCDD	0.106	10 & 11	30	252.37	256.46	12.6	0.420		3,180	8.95	130
1,2,3,4,6,7,8-HpCDD	1.33	10 & 11	30	252.37	256.46	158	5.270		39,900	8.95	1,140
2,3,4,7,8-PeCDF	0.0397	10 & 11	30	252.37	256.46	4.72	0.157		1,191	8.95	90.1
2,3,7,8-TCDD	0.128	12 & 13	30	262.33	281.82	14.6	0.488		3,840	9.60	58.4
1,2,3,7,8-PeCDD	0.0740	12 & 13	30	262.33	281.82	8.46	0.282		2,220	9.60	62.6
1,2,3,6,7,8-HxCDD	0.106	12 & 13	30	262.33	281.82	12.1	0.404		3,180	9.60	130
1,2,3,4,6,7,8-HpCDD	1.33	12 & 13	30	262.33	281.82	152	5.070		39,900	9.60	1,160
2,3,4,7,8-PeCDF	0.0397	12 & 13	30	262.33	281.82	4.54	0.151		1,191	9.60	87.5
2,3,7,8-TCDD	0.128	14 & 15	30	245.17	250.93	15.7	0.522		3,840	9.00	62.4
1,2,3,7,8-PeCDD	0.0740	14 & 15	30	245.17	250.93	9.06	0.302		2,220	9.00	68.4
1,2,3,6,7,8-HxCDD	0.106	14 & 15	30	245.17	250.93	13.0	0.432		3,180	9.00	138
1,2,3,4,6,7,8-HpCDD	1.33	14 & 15	30	245.17	250.93	163	5.425		39,900	9.00	1,190
2,3,4,7,8-PeCDF	0.0397	14 & 15	30	245.17	250.93	4.86	0.162		1,191	9.00	98.6
2,3,7,8-TCDD	0.128	16 & 17	30	246.37	254.55	15.6	0.520		3,840	8.35	57.0
1,2,3,7,8-PeCDD	0.0740	16 & 17	30	246.37	254.55	9.01	0.300		2,220	8.35	69.3
1,2,3,6,7,8-HxCDD	0.106	16 & 17	30	246.37	254.55	12.9	0.430		3,180	8.35	137
1,2,3,4,6,7,8-HpCDD	1.33	16 & 17	30	246.37	254.55	162	5.398		39,900	8.35	1,260
2,3,4,7,8-PeCDF	0.0397	16 & 17	30	246.37	254.55	4.83	0.161		1,191	8.35	104
2,3,7,8-TCDD	0.128	18 & 19	30	247.63	249.86	15.5	0.517		3,840	8.31	65.4
1,2,3,7,8-PeCDD	0.0740	18 & 19	30	247.63	249.86	8.96	0.299		2,220	8.31	70.2
1,2,3,6,7,8-HxCDD	0.106	18 & 19	30	247.63	249.86	12.8	0.428		3,180	8.31	143
1,2,3,4,6,7,8-HpCDD	1.33	18 & 19	30	247.63	249.86	161	5.371		39,900	8.31	1,240
2,3,4,7,8-PeCDF	0.0397	18 & 19	30	247.63	249.86	4.81	0.160		1,191	8.31	99.4

Table D-5. (cont.)

	Midland Reference Gavage (Group 1)											
			Using Terminal BW			Fraction Retained in Liver		Fraction Retained in Fat		Fraction Retained Liver+Fat		
Analyte	WHO TEF (unitless)	Liver TEQ (pg/g)	Fat Weight Fraction (w_a) (unitless)	Fat Weight (g)	Fat Conc. (pg/g)	FR_{liver} (unitless)	FR_{liver} S.D.	FR_{fat} (unitless)	FR_{fat} S.D.	FR_{sum} (unitless)	FR_{sum} S.D.	
2,3,7,8-TCDD	1					0.139	0.009	0.319	0.017	0.458	0.020	
1,2,3,7,8-PeCDD	1					0.265	0.009	0.250	0.016	0.515	0.013	
1,2,3,6,7,8-HxCDD	0.1					0.376	0.015	0.117	0.011	0.493	0.014	
1,2,3,4,6,7,8-HpCDD	0.01					0.265	0.009	0.041	0.005	0.306	0.012	
2,3,4,7,8-PeCDF	0.5					0.710	0.027	0.086	0.008	0.796	0.022	
2,3,7,8-TCDD	1	59.7	0.0677	17.36	72.5	0.139		0.328		0.467		
1,2,3,7,8-PeCDD	1	63.4	0.0677	17.36	33.6	0.256		0.263		0.518		
1,2,3,6,7,8-HxCDD	0.1	13	0.0677	17.36	21.8	0.366		0.119		0.485		
1,2,3,4,6,7,8-HpCDD	0.01	11.4	0.0677	17.36	93.1	0.256		0.040		0.296		
2,3,4,7,8-PeCDF	0.5	45.05	0.0677	17.36	6.75 *J*	0.677		0.098		0.775		
2,3,7,8-TCDD	1	58.4	0.0727	20.49	64.1	0.146		0.342		0.488		
1,2,3,7,8-PeCDD	1	62.6	0.0727	20.49	28.7	0.271		0.265		0.536		
1,2,3,6,7,8-HxCDD	0.1	13	0.0727	20.49	19.4	0.392		0.125		0.517		
1,2,3,4,6,7,8-HpCDD	0.01	11.6	0.0727	20.49	92.9	0.279		0.048		0.327		
2,3,4,7,8-PeCDF	0.5	43.75	0.0727	20.49	5.13 *J*	0.705		0.088		0.794		
2,3,7,8-TCDD	1	62.4	0.0666	16.71	70.9	0.146		0.308		0.455		
1,2,3,7,8-PeCDD	1	68.4	0.0666	16.71	30.0	0.277		0.226		0.503		
1,2,3,6,7,8-HxCDD	0.1	13.8	0.0666	16.71	19.0	0.391		0.100		0.490		
1,2,3,4,6,7,8-HpCDD	0.01	11.9	0.0666	16.71	80.1	0.268		0.034		0.302		
2,3,4,7,8-PeCDF	0.5	49.3	0.0666	16.71	5.48 *J*	0.745		0.077		0.822		
2,3,7,8-TCDD	1	57	0.0673	17.13	71.7	0.124		0.320		0.444		
1,2,3,7,8-PeCDD	1	69.3	0.0673	17.13	32.8	0.261		0.253		0.514		
1,2,3,6,7,8-HxCDD	0.1	13.7	0.0673	17.13	23.5	0.360		0.127		0.486		
1,2,3,4,6,7,8-HpCDD	0.01	12.6	0.0673	17.13	103	0.264		0.044		0.308		
2,3,4,7,8-PeCDF	0.5	52	0.0673	17.13	6.03 *J*	0.729		0.087		0.816		
2,3,7,8-TCDD	1	65.4	0.0664	16.58	68.7	0.142		0.297		0.438		
1,2,3,7,8-PeCDD	1	70.2	0.0664	16.58	32.5	0.263		0.243		0.506		
1,2,3,6,7,8-HxCDD	0.1	14.3	0.0664	16.58	22.0	0.374		0.115		0.488		
1,2,3,4,6,7,8-HpCDD	0.01	12.4	0.0664	16.58	96.0	0.258		0.040		0.298		
2,3,4,7,8-PeCDF	0.5	49.7	0.0664	16.58	5.83 *J*	0.694		0.081		0.775		

Table D-6. Tissue concentrations, doses, and RBA calculations for the rat pilot study: Tittabawassee River flood plain soil

	Tittabawassee River Flood Plain Soil (Group 4)											
	Soil THT02769/Diet Blend (Test Article #2)						Using Mean BW					
Analyte	Mean Conc. (pg/g)	% of TEQ in soil)	Group 4 Rat IDs	Total Feed Intake (g)	Mean BW (g)	Terminal BW (g)	Total Dose (pg/g BW)	Avg. Daily Dose (pg/g BW/d)	Avg. Daily Dose S.D.	Total Dose (pg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)
2,3,7,8-TCDF	83.7	25.4%	Grp 4 Mean	579.34	251.85	263.81	193	6.425	0.372	48,491		
1,2,3,7,8-PeCDF	51.0	6.3%	Grp 4 Mean	579.34	251.85	263.81	117	3.915	0.227	29,546		
2,3,4,7,8-PeCDF	43.9	52.1%	Grp 4 Mean	579.34	251.85	263.81	101	3.370	0.195	25,433		
1,2,3,4,7,8-HxCDF	34.2	8.5%	Grp 4 Mean	579.34	251.85	263.81	78.7	2.625	0.152	19,813		
1,2,3,6,7,8-HxCDF	8.45	1.9%	Grp 4 Mean	579.34	251.85	263.81	19.4	0.649	0.038	4,895		
1,2,3,6,7,8-HxCDF (excluding outlier)[a]												
2,3,7,8-TCDF	83.7	25.4%	40 & 41	548.74	251.24	259.24	183	6.094		45,929	9.11	316
1,2,3,7,8-PeCDF	51.0	6.3%	40 & 41	548.74	251.24	259.24	111	3.713		27,985	9.11	254
2,3,4,7,8-PeCDF	43.9	52.1%	40 & 41	548.74	251.24	259.24	95.9	3.196		24,089	9.11	1,050
1,2,3,4,7,8-HxCDF	34.2	8.5%	40 & 41	548.74	251.24	259.24	74.7	2.490		18,767	9.11	641
1,2,3,6,7,8-HxCDF	8.45	1.9%	40 & 41	548.74	251.24	259.24	18.5	0.615		4,637	9.11	161
2,3,7,8-TCDF	83.7	25.4%	42 & 43	592.14	251.77	274.75	197	6.562		49,562	10.76	333
1,2,3,7,8-PeCDF	51.0	6.3%	42 & 43	592.14	251.77	274.75	120	3.998		30,199	10.76	258
2,3,4,7,8-PeCDF	43.9	52.1%	42 & 43	592.14	251.77	274.75	103	3.442		25,995	10.76	944
1,2,3,4,7,8-HxCDF	34.2	8.5%	42 & 43	592.14	251.77	274.75	80.4	2.681		20,251	10.76	590
1,2,3,6,7,8-HxCDF	8.45	1.9%	42 & 43	592.14	251.77	274.75	19.9	0.662		5,004	10.76	151
2,3,7,8-TCDF	83.7	25.4%	44 & 45	564.56	263.97	277.66	179	5.967		47,254	9.79	342
1,2,3,7,8-PeCDF	51.0	6.3%	44 & 45	564.56	263.97	277.66	109	3.636		28,793	9.79	266
2,3,4,7,8-PeCDF	43.9	52.1%	44 & 45	564.56	263.97	277.66	93.9	3.130		24,784	9.79	1,080
1,2,3,4,7,8-HxCDF	34.2	8.5%	44 & 45	564.56	263.97	277.66	73.1	2.438		19,308	9.79	667
1,2,3,6,7,8-HxCDF	8.45	1.9%	44 & 45	564.56	263.97	277.66	18.1	0.602		4,771	9.79	175
2,3,7,8-TCDF	83.7	25.4%	46 & 47	605.22	250.95	258.63	202	6.729		50,657	8.31	360
1,2,3,7,8-PeCDF	51.0	6.3%	46 & 47	605.22	250.95	258.63	123	4.100		30,866	8.31	291
2,3,4,7,8-PeCDF	43.9	52.1%	46 & 47	605.22	250.95	258.63	106	3.529		26,569	8.31	1,190
1,2,3,4,7,8-HxCDF	34.2	8.5%	46 & 47	605.22	250.95	258.63	82.5	2.749		20,699	8.31	733
1,2,3,6,7,8-HxCDF	8.45	1.9%	46 & 47	605.22	250.95	258.63	20.4	0.679		5,114	8.31	697 [a]
2,3,7,8-TCDF	83.7	25.4%	48 & 49	586.04	241.30	248.76	203	6.776		49,052	8.49	341
1,2,3,7,8-PeCDF	51.0	6.3%	48 & 49	586.04	241.30	248.76	124	4.129		29,888	8.49	275
2,3,4,7,8-PeCDF	43.9	52.1%	48 & 49	586.04	241.30	248.76	107	3.554		25,727	8.49	1,160
1,2,3,4,7,8-HxCDF	34.2	8.5%	48 & 49	586.04	241.30	248.76	83.1	2.769		20,043	8.49	711
1,2,3,6,7,8-HxCDF	8.45	1.9%	48 & 49	586.04	241.30	248.76	20.5	0.684		4,952	8.49	180

Table D-6. (cont.)

	Tittabawassee River Flood Plain Soil (Group 4)											
			Using Terminal BW			Fraction Retained		Fraction Retained		Fraction Retained		RBA Grp 4: Grp 2
			Fat Weight									Indiv: Grp Mean
	WHO	Liver	Fraction	Fat	Fat	in Liver	FR_{liver}	in Fat	FR_{fat}	Liver+Fat	FR_{sum}	Using FR_{sum}
Analyte	TEF (unitless)	TEQ (pg/g)	(w_a) (unitless)	Weight (g)	Conc. (pg/g)	FR_{liver} (unitless)	S.D.	FR_{fat} (unitless)	S.D.	FR_{sum} (unitless)	S.D.	(unitless)
2,3,7,8-TCDF	0.1					0.065	0.006	0.049	0.010	0.114	0.015	89%
1,2,3,7,8-PeCDF	0.05					0.084	0.007	0.032	0.005	0.117	0.010	58%
2,3,4,7,8-PeCDF	0.5					0.394	0.021	0.031	0.004	0.425	0.022	52%
1,2,3,4,7,8-HxCDF	0.1					0.312	0.017	0.029	0.003	0.341	0.017	57%
1,2,3,6,7,8-HxCDF	0.1					0.488	0.361	0.028	0.003	0.516	0.362	82%
1,2,3,6,7,8-HxCDF (excluding outlier)[a]						0.327[a]	0.022[a]			0.355[a]	0.024[a]	56%[a]
2,3,7,8-TCDF	0.1	31.6	0.0682	17.69	132	0.063		0.051		0.114		0.894
1,2,3,7,8-PeCDF	0.05	12.7	0.0682	17.69	54.3	0.083		0.034		0.117		0.580
2,3,4,7,8-PeCDF	0.5	525	0.0682	17.69	45.1	0.397		0.033		0.430		0.530
1,2,3,4,7,8-HxCDF	0.1	64.1	0.0682	17.69	32.1	0.311		0.030		0.341		0.570
1,2,3,6,7,8-HxCDF	0.1	16.1	0.0682	17.69	7.73 *J*	0.316		0.029		0.346		0.547
2,3,7,8-TCDF	0.1	33.3	0.0713	19.59	140	0.072		0.055		0.128		1.005
1,2,3,7,8-PeCDF	0.05	12.9	0.0713	19.59	52.1	0.092		0.034		0.126		0.624
2,3,4,7,8-PeCDF	0.5	472	0.0713	19.59	41.3	0.391		0.031		0.422		0.520
1,2,3,4,7,8-HxCDF	0.1	59	0.0713	19.59	28.9	0.313		0.028		0.341		0.570
1,2,3,6,7,8-HxCDF	0.1	15.1	0.0713	19.59	6.51 *J*	0.325		0.025		0.350		0.554
2,3,7,8-TCDF	0.1	34.2	0.0719	19.96	133	0.071		0.056		0.127		1.000
1,2,3,7,8-PeCDF	0.05	13.3	0.0719	19.96	51.2	0.090		0.035		0.126		0.625
2,3,4,7,8-PeCDF	0.5	540	0.0719	19.96	42.7	0.427		0.034		0.461		0.568
1,2,3,4,7,8-HxCDF	0.1	66.7	0.0719	19.96	30.2	0.338		0.031		0.369		0.616
1,2,3,6,7,8-HxCDF	0.1	17.5	0.0719	19.96	7.22 *J*	0.359		0.030		0.389		0.615
2,3,7,8-TCDF	0.1	36	0.0681	17.61	141	0.059		0.049		0.108		0.851
1,2,3,7,8-PeCDF	0.05	14.55	0.0681	17.61	61.3	0.078		0.035		0.113		0.562
2,3,4,7,8-PeCDF	0.5	595	0.0681	17.61	50.2	0.372		0.033		0.405		0.500
1,2,3,4,7,8-HxCDF	0.1	73.3	0.0681	17.61	37.7	0.294		0.032		0.326		0.545
1,2,3,6,7,8-HxCDF	0.1	69.7	0.0681	17.61	8.64 *J*	1.133[a]		0.030		1.162[a]		1.837[a]
2,3,7,8-TCDF	0.1	34.1	0.0661	16.45	97.4	0.059		0.033		0.092		0.722
1,2,3,7,8-PeCDF	0.05	13.75	0.0661	16.45	43.2	0.078		0.024		0.102		0.505
2,3,4,7,8-PeCDF	0.5	580	0.0661	16.45	39.5	0.383		0.025		0.408		0.503
1,2,3,4,7,8-HxCDF	0.1	71.1	0.0661	16.45	31.2	0.301		0.026		0.327		0.545
1,2,3,6,7,8-HxCDF	0.1	18	0.0661	16.45	7.42 *J*	0.309		0.025		0.333		0.527

Table D-6. (cont.)

	Tittabawassee River Flood Plain Soil Reference Gavage (Group 2)										
	Reference Mixture #2					Using Mean BW					
Analyte	Mean Conc. (ng/mL)	Group 2 Rat IDs	Total Gavage Volume (mL)	Mean BW (g)	Terminal BW (g)	Total Dose (pg/g BW)	Avg. Daily Dose (pg/g BW/d)	Avg. Daily Dose S.D.	Total Dose (pg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)
2,3,7,8-TCDF	2.35	Grp 2 Mean	30 [b]	245.12 [b]	251.20 [b]	288	8.808	1.753	70,500		
1,2,3,7,8-PeCDF	1.17	Grp 2 Mean	30 [b]	245.12 [b]	251.20 [b]	143	4.385	0.873	35,100		
2,3,4,7,8-PeCDF	0.954	Grp 2 Mean	30 [b]	245.12 [b]	251.20 [b]	117	3.576	0.711	28,620		
1,2,3,4,7,8-HxCDF	0.808	Grp 2 Mean	30 [b]	245.12 [b]	251.20 [b]	98.9	3.029	0.603	24,240		
1,2,3,6,7,8-HxCDF	0.212	Grp 2 Mean	30 [b]	245.12 [b]	251.20 [b]	25.9	0.795	0.158	6,360		
1,2,3,6,7,8-HxCDF (excluding outlier)[a]											
2,3,7,8-TCDF	2.35	20 & 21	30	241.04	247.42	292	9.750		70,500	8.44	577
1,2,3,7,8-PeCDF	1.17	20 & 21	30	241.04	247.42	146	4.854		35,100	8.44	588
2,3,4,7,8-PeCDF	0.954	20 & 21	30	241.04	247.42	119	3.958		28,620	8.44	2,450
1,2,3,4,7,8-HxCDF	0.808	20 & 21	30	241.04	247.42	101	3.352		24,240	8.44	1,570
1,2,3,6,7,8-HxCDF	0.212	20 & 21	30	241.04	247.42	26.4	0.880		6,360	8.44	445
2,3,7,8-TCDF	2.35	22 & 23	30	244.28	252.49	289	9.620		70,500	8.67	556
1,2,3,7,8-PeCDF	1.17	22 & 23	30	244.28	252.49	144	4.790		35,100	8.67	530
2,3,4,7,8-PeCDF	0.954	22 & 23	30	244.28	252.49	117	3.905		28,620	8.67	2,370
1,2,3,4,7,8-HxCDF	0.808	22 & 23	30	244.28	252.49	99.2	3.308		24,240	8.67	1,470
1,2,3,6,7,8-HxCDF	0.212	22 & 23	30	244.28	252.49	26.0	0.868		6,360	8.67	399
2,3,7,8-TCDF	2.35	24 & 29	17.5 [b]	241.20 [b]	235.56 [b]	171	5.683		41,125	8.97	450
1,2,3,7,8-PeCDF	1.17	24 & 29	17.5 [b]	241.20 [b]	235.56 [b]	84.9	2.830		20,475	8.97	468
2,3,4,7,8-PeCDF	0.954	24 & 29	17.5 [b]	241.20 [b]	235.56 [b]	69.2	2.307		16,695	8.97	1,480
1,2,3,4,7,8-HxCDF	0.808	24 & 29	17.5 [b]	241.20 [b]	235.56 [b]	58.6	1.954		14,140	8.97	958
1,2,3,6,7,8-HxCDF	0.212	24 & 29	17.5 [b]	241.20 [b]	235.56 [b]	15.4	0.513		3,710	8.97	261
2,3,7,8-TCDF	2.35	25 & 26	30	251.07	253.67	281	9.360		70,500	8.26	632
1,2,3,7,8-PeCDF	1.17	25 & 26	30	251.07	253.67	140	4.660		35,100	8.26	633
2,3,4,7,8-PeCDF	0.954	25 & 26	30	251.07	253.67	114	3.800		28,620	8.26	2,670
1,2,3,4,7,8-HxCDF	0.808	25 & 26	30	251.07	253.67	96.5	3.218		24,240	8.26	1,580
1,2,3,6,7,8-HxCDF	0.212	25 & 26	30	251.07	253.67	25.3	0.844		6,360	8.26	441
2,3,7,8-TCDF	2.35	27 & 28	30	244.09	251.25	289	9.628		70,500	8.54	632
1,2,3,7,8-PeCDF	1.17	27 & 28	30	244.09	251.25	144	4.793		35,100	8.54	603
2,3,4,7,8-PeCDF	0.954	27 & 28	30	244.09	251.25	117	3.908		28,620	8.54	2,650
1,2,3,4,7,8-HxCDF	0.808	27 & 28	30	244.09	251.25	99.3	3.310		24,240	8.54	1,610
1,2,3,6,7,8-HxCDF	0.212	27 & 28	30	244.09	251.25	26.1	0.869		6,360	8.54	462

Table D-6. (cont.)

	Tittabawassee River Flood Plain Soil Reference Gavage (Group 2)										
			Using Terminal BW			Fraction Retained		Fraction Retained		Fraction Retained	
			Fat Weight								
	WHO	Liver	Fraction	Fat	Fat	in Liver[b]		in Fat[b]		Liver+Fat[b]	
Analyte	TEF (unitless)	TEQ (pg/g)	(w_a) (unitless)	Weight (g)	Conc. (pg/g)	FR_{liver} (unitless)	FR_{liver} S.D.	FR_{fat} (unitless)	FR_{fat} S.D.	FR_{sum} (unitless)	FR_{sum} S.D.
2,3,7,8-TCDF	0.1					0.072	0.004	0.055	0.003	0.127	0.006
1,2,3,7,8-PeCDF	0.05					0.142	0.008	0.060	0.007	0.202	0.014
2,3,4,7,8-PeCDF	0.5					0.750	0.036	0.061	0.007	0.811	0.040
1,2,3,4,7,8-HxCDF	0.1					0.545	0.017	0.055	0.008	0.599	0.020
1,2,3,6,7,8-HxCDF	0.1					0.582	0.032	0.051	0.007	0.633	0.034
1,2,3,6,7,8-HxCDF (excluding outlier)[a]											
2,3,7,8-TCDF	0.1	57.7	0.0659	16.30	233	0.069		0.054		0.123	
1,2,3,7,8-PeCDF	0.05	29.4	0.0659	16.30	129	0.141		0.060		0.201	
2,3,4,7,8-PeCDF	0.5	1225	0.0659	16.30	103	0.723		0.059		0.781	
1,2,3,4,7,8-HxCDF	0.1	157	0.0659	16.30	82.4	0.547		0.055		0.602	
1,2,3,6,7,8-HxCDF	0.1	44.5	0.0659	16.30	20.9	0.591		0.054		0.644	
2,3,7,8-TCDF	0.1	55.6	0.0669	16.89	219	0.068		0.052		0.121	
1,2,3,7,8-PeCDF	0.05	26.5	0.0669	16.89	110	0.131		0.053		0.184	
2,3,4,7,8-PeCDF	0.5	1185	0.0669	16.89	92.0	0.718		0.054		0.772	
1,2,3,4,7,8-HxCDF	0.1	147	0.0669	16.89	66.8	0.526		0.047		0.572	
1,2,3,6,7,8-HxCDF	0.1	39.9	0.0669	16.89	16.0	0.544		0.042		0.586	
2,3,7,8-TCDF	0.1	45	0.0635	14.96	264	0.098		0.096		0.194	
1,2,3,7,8-PeCDF	0.05	23.4	0.0635	14.96	119	0.205		0.087		0.292	
2,3,4,7,8-PeCDF	0.5	740	0.0635	14.96	69.6	0.795		0.062		0.858	
1,2,3,4,7,8-HxCDF	0.1	95.8	0.0635	14.96	50.8	0.608		0.054		0.661	
1,2,3,6,7,8-HxCDF	0.1	26.1	0.0635	14.96	13.2 *J*	0.631		0.053		0.684	
2,3,7,8-TCDF	0.1	63.2	0.0671	17.03	244	0.074		0.059		0.133	
1,2,3,7,8-PeCDF	0.05	31.65	0.0671	17.03	141	0.149		0.068		0.217	
2,3,4,7,8-PeCDF	0.5	1335	0.0671	17.03	119	0.771		0.071		0.841	
1,2,3,4,7,8-HxCDF	0.1	158	0.0671	17.03	91.9	0.538		0.065		0.603	
1,2,3,6,7,8-HxCDF	0.1	44.1	0.0671	17.03	22.1	0.573		0.059		0.632	
2,3,7,8-TCDF	0.1	63.2	0.0666	16.74	230	0.077		0.055		0.131	
1,2,3,7,8-PeCDF	0.05	30.15	0.0666	16.74	120	0.147		0.057		0.204	
2,3,4,7,8-PeCDF	0.5	1325	0.0666	16.74	100	0.791		0.058		0.849	
1,2,3,4,7,8-HxCDF	0.1	161	0.0666	16.74	75.8	0.567		0.052		0.620	
1,2,3,6,7,8-HxCDF	0.1	46.2	0.0666	16.74	18.2	0.620		0.048		0.668	

[a] Excluding outlier.

[b] Group means exclude results from rat pair (24 & 29), which were sacrificed early.

Table D-7. Swine body weights during the pilot study

	Body Weight (kg)											
Swine ID	Day -1 (10/4/04)	Day 2 (10/7/04)	Day 5 (10/10/04)	Day 8 (10/13/04)	Day 11 (10/16/04)	Day 14 (10/19/04)	Day 17 (10/22/04)	Day 21 (10/25/04)	Day 24 (10/29/04)	Day 27 (10/31/04)	Day 30 (11/3/04)	Avgerage Day -1 to 30
Group 1: Midland Reference Oil												
415	11.20	12.55	13.70	15.25	16.40	18.20	20.00	22.45	24.20	26.05	28.55	18.96
419	12.50	13.80	14.75	15.95	17.55	19.65	21.40	23.35	25.75	28.15	30.40	20.30
435	11.30	12.35	13.65	15.30	16.20	17.90	19.15	20.90	22.55	24.15	26.35	18.16
439	11.40	12.50	13.90	15.50	16.55	18.60	20.10	21.80	23.95	25.90	28.30	18.95
443	11.90	13.35	14.85	16.70	18.15	19.95	21.30	23.45	25.20	27.60	29.25	20.15
Grp 1 Mean	**11.66**	**12.91**	**14.17**	**15.74**	**16.97**	**18.86**	**20.39**	**22.39**	**24.33**	**26.37**	**28.57**	**19.31**
Group 2: Tittabawassee River Flood Plain Soil Reference Oil												
403	10.75	11.80	13.00	14.00	15.40	17.25	18.90	20.75	22.75	24.45	26.90	17.81
410	10.60	11.90	12.95	14.50	15.90	17.50	19.20	20.80	22.80	23.95	26.15	17.84
425	11.75	13.00	14.10	15.20	16.85	18.25	20.00	21.40	23.50	25.80	27.80	18.88
432	10.80	11.95	13.65	15.10	16.50	18.50	20.05	21.90	23.85	26.05	28.40	18.80
447	10.30	11.55	12.50	13.85	15.40	17.05	18.95	20.60	21.85	24.80	26.60	17.59
Grp 2 Mean	**10.84**	**12.04**	**13.24**	**14.53**	**16.01**	**17.71**	**19.42**	**21.09**	**22.95**	**25.01**	**27.17**	**18.18**
Group 3: Midland Soil												
405	10.30	11.45	13.00	14.35	16.15	17.85	19.75	21.40	23.10	25.50	27.85	18.25
407	11.65	13.00	14.45	16.15	17.60	19.40	21.40	23.65	25.05	27.30	29.25	19.90
417	10.45	12.00	13.30	15.00	16.35	17.95	19.75	21.30	23.20	25.40	27.60	18.39
418	11.50	12.70	14.10	15.40	16.80	18.20	19.60	21.75	23.05	25.05	26.75	18.63
436	11.05	12.35	13.75	15.05	16.50	18.05	19.95	21.75	24.10	26.30	28.50	18.85
Grp 3 Mean	**10.99**	**12.30**	**13.72**	**15.19**	**16.68**	**18.29**	**20.09**	**21.97**	**23.70**	**25.91**	**27.99**	**18.80**
Tittabawassee River Flood Plain Soil (Group 4)												
427	12.40	13.70	15.10	16.50	18.25	19.90	22.30	23.60	25.65	27.25	29.70	20.40
428	11.00	12.70	13.80	15.10	16.45	18.40	19.65	21.50	23.70	25.50	27.60	18.67
440	11.05	12.25	13.70	15.20	16.65	18.60	20.10	21.90	23.75	25.60	28.00	18.80
441	11.95	13.35	14.35	15.35	16.55	18.40	19.90	21.55	23.55	25.60	27.90	18.95
444	11.20	12.05	13.45	14.80	16.25	18.20	19.55	21.00	22.00			16.50[a]
Grp 4 Mean	**11.52**	**12.81**	**14.08**	**15.39**	**16.83**	**18.70**	**20.30**	**21.91**	**23.73**	**25.99**	**28.30**	**19.20[a]**
Body Composition Group												
401	11.90	13.30	14.40	15.95	17.30	18.85	20.35	22.05	23.90	25.75	28.05	19.25
402	11.00	12.50	13.85	15.65	16.85	18.95	20.85	22.75	24.90	27.30	29.65	19.48
413	12.30	13.10	14.45	15.75	17.55	19.30	20.90	23.30	25.35	27.95	31.30	20.11

[a] Swine #444 became ill and died early. Group means exclude results associated with this animal.

Table D-8. Swine necropsy liver and fat sample weights

Swine ID	Liver Weight (g)	Abdominal Fat Sample Weight (g)
Group 1: Midland Reference Oil		
415	594.8	50.40
419	754.6	54.60
435	500.8	46.58
439	660.8	64.56
443	655.7	55.47
Grp 1 Mean	**633.3**	**54.32**
Group 2: Tittabawassee River Flood Plain Soil Reference Oil		
403	621.4	38.90
410	568.5	52.75
425	560.1	53.80
432	572.7	53.72
447	601.0	50.66
Grp 2 Mean	**584.7**	**49.97**
Group 3: Midland Soil		
405	716.3	62.42
407	715.6	48.20
417	757.1	51.18
418	728.9	53.00
436	738.6	50.02
Grp 3 Mean	**731.3**	**52.96**
Tittabawassee River Flood Plain Soil (Group 4)		
427	566.9	50.77
428	656.1	48.17
440	795.7	50.89
441	646.0	47.74
444[a]	533.2	5.20
Grp 4 Mean	**666.2** [a]	**49.39** [a]

Notes:

Fat was taken from the abdominal cavity. Liver (gallbladder removed) was weighed and then sample for MROD was taken from 3 different areas in the liver, minced with a knife and scissors on a clean glass plate and packed into a 5ml cryovial and frozen in liquid N2. After this sample was taken, the liver was wrapped in foil, placed in a zipper-sealed freezer bag and frozen at -80 °C.

Fat was stripped from between the skin and the abdominal wall.

Fat removal was very time consuming. Pigs this age have little fat.

[a] Swine #444 became ill and died early. Group means exclude results associated with this animal.

Table D-9. Swine body composition data

Swine ID	Dead Weight (g)	Carcass Weight[a] (g)	Percent Dressed[b] (%)	Skin Weight (g)	Subcutaneous Fat Weight (g)	Seam Fat Weight (g)	Leaf Fat Weight (g)	Muscle Weight (g)	Total Fat Weight (g)	Percent Fat (%)	Percent Muscle (%)	Percent Skin (%)
401	28,770	21,092.4	73.31	1,528.3	1,229.0	140.6	62.3	11,157.4	1,431.9	6.79	52.90	7.25
402	28,770	22,453.2	78.04	1,684.8	1,274.7	268.0	77.6	12,940.4	1,620.3	7.22	57.63	7.50
413	31,020	22,680.0	73.11	1,697.4	1,086.7	253.7	69.8	12,475.6	1,410.2	6.22	55.01	7.48

[a] Weight after removing intestinal contents.

[b] Carcass weight as a percentage of dead weight.

Table D-10. Swine liver microsomal EROD and MROD activities

Group	Entrix Sample ID	Exponent Swine ID	EROD (pmol/mg/min)	MROD (pmol/mg/min)
1	ESL-5	415	26.1	143
1	ESL-8	419	37.4	106
1	ESL-13	435	3.91	39.8
1	ESL-15	439	14.9	41.1
1	ESL-18	443	43.9	147.6
2	ESL-1	403	31.5	103.4
2	ESL-4	410	33.0	161
2	ESL-9	425	38.3	169
2	ESL-12	432	34.6	83.8
2	ESL-20	447	38.5	96.7
3	ERL-2	405	27.3	83.7
3	ESL-3	407	19.8	93.8
3	ESL-6	417	24.4	132
3	ESL-7	418	26.9	138
3	ESL-14	436	25.7	124
4	ESL-10	427	28.0	87.0
4	ESL-11	428	21.2	87.0
4	ESL-16	440	15.3	81.6
4	ESL-17	441	47.1	130.5
4	ESL-19	444[a]	11.6	28.9

Note: All assays conducted as outlined in SOP250 MSU-ATL SOP 250 version 1

[a] Results excluded from analyses because this animal died before end of study.

Table D-11. Tissue concentrations, doses, and RBA calculations for the swine pilot study: Midland soil

	Midland Soil (Group 3)																
	Dow Corporate Center (CC-S-27)					Using Mean BW						Fat Weight				Using	Using
Analyte	Soil Mean Conc.[a] (pg/g)	% of TEQ	Soil Dose Daily Mass of Chemical (ng/day)	Pig ID	Total Dose (ng)	Average Daily Dose (ng/kg BW/d)	Average Daily Dose S.D.	Mean Body Weight (kg)	Terminal Body Weight (kg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)	Using term. BW (g)	Fat Conc. (pg/g)	WHO TEF (unitless)	Using 1/2 DL Liver TEQ (pg/g)	DL Liver TEQ (pg/g)
2,3,7,8-TCDD	131	49%	1.31	Grp 3 Mean	39.4	0.0699	0.0024	18.80	27.99	731.3		1,887		1		
1,2,3,7,8-PeCDD	66.9	25%	0.669	Grp 3 Mean	20.1	0.0356	0.0012	18.80	27.99	731.3		1,887		1		
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	Grp 3 Mean	22.1	0.0391	0.0013	18.80	27.99	731.3		1,887		0.1		
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	Grp 3 Mean	350	0.621	0.021	18.80	27.99	731.3		1,887		0.01		
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	Grp 3 Mean	10.8	0.0192	0.0006	18.80	27.99	731.3		1,887		0.5		
2,3,7,8-TCDD	131	49%	1.31	405	39.4	0.072		18.25	27.85	716.3	0.200 *J*	1,877	0.508 *Um*	1	0.200	0.200
1,2,3,7,8-PeCDD	66.9	25%	0.669	405	20.1	0.037		18.25	27.85	716.3	0.195 *U*	1,877	0.443 *Um*	1	0.098	0.195
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	405	22.1	0.040		18.25	27.85	716.3	0.401 *U*	1,877	0.500 *U*	0.1	0.020	0.040
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	405	350	0.639		18.25	27.85	716.3	5.17	1,877	5.62	0.01	0.052	0.052
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	405	10.8	0.020		18.25	27.85	716.3	0.425 *J*	1,877	0.390 *U*	0.5	0.213	0.213
2,3,7,8-TCDD	131	49%	1.31	407	39.4	0.066		19.90	29.25	715.6	0.224 *J*	1,971	0.638 *Um*	1	0.224	0.224
1,2,3,7,8-PeCDD	66.9	25%	0.669	407	20.1	0.034		19.90	29.25	715.6	0.232 *J*	1,971	0.611 *Um*	1	0.232	0.232
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	407	22.1	0.037		19.90	29.25	715.6	0.408 *J*	1,971	0.956 *J*	0.1	0.041	0.041
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	407	350	0.586		19.90	29.25	715.6	12.0	1,971	7.67	0.01	0.120	0.120
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	407	10.8	0.018		19.90	29.25	715.6	0.856 *J*	1,971	0.308 *Um*	0.5	0.428	0.428
2,3,7,8-TCDD	131	49%	1.31	417	39.4	0.071		18.39	27.60	757.1	0.174 *U*	1,860	0.773 *J*	1	0.087	0.174
1,2,3,7,8-PeCDD	66.9	25%	0.669	417	20.1	0.036		18.39	27.60	757.1	0.120 *U*	1,860	0.552 *J*	1	0.060	0.120
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	417	22.1	0.040		18.39	27.60	757.1	0.225 *Um*	1,860	0.833 *Um*	0.1	0.011	0.023
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	417	350	0.634		18.39	27.60	757.1	6.81	1,860	8.15	0.01	0.068	0.068
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	417	10.8	0.020		18.39	27.60	757.1	0.558 *J*	1,860	0.303 *Um*	0.5	0.279	0.279
2,3,7,8-TCDD	131	49%	1.31	418	39.4	0.071		18.63	26.75	728.9	0.284 *J*	1,803	0.805 *J*	1	0.284	0.284
1,2,3,7,8-PeCDD	66.9	25%	0.669	418	20.1	0.036		18.63	26.75	728.9	0.189 *U*	1,803	0.740 *J*	1	0.095	0.189
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	418	22.1	0.039		18.63	26.75	728.9	0.268 *Um*	1,803	1.39 *J*	0.1	0.013	0.027
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	418	350	0.626		18.63	26.75	728.9	8.46	1,803	11.4	0.01	0.085	0.085
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	418	10.8	0.019		18.63	26.75	728.9	0.600 *J*	1,803	0.504 *J*	0.5	0.300	0.300
2,3,7,8-TCDD	131	49%	1.31	436	39.4	0.070		18.85	28.50	738.6	0.248 *J*	1,921	0.814 *J*	1	0.248	0.248
1,2,3,7,8-PeCDD	66.9	25%	0.669	436	20.1	0.035		18.85	28.50	738.6	0.208 *Um*	1,921	0.677 *Um*	1	0.104	0.208
1,2,3,6,7,8-HxCDD	73.5	2.7%	0.735	436	22.1	0.039		18.85	28.50	738.6	0.402 *Um*	1,921	1.25 *J*	0.1	0.020	0.040
1,2,3,4,6,7,8-HpCDD	1,167	4.3%	11.7	436	350	0.619		18.85	28.50	738.6	11.9	1,921	9.81	0.01	0.119	0.119
2,3,4,7,8-PeCDF	36.1	6.7%	0.361	436	10.8	0.019		18.85	28.50	738.6	0.816 *J*	1,921	0.436 *Um*	0.5	0.408	0.408

Table D-11. (cont.)

	Midland Soil (Group 3)													
	Using 1/2 DL							Using DL						
Analyte	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.	RBA Grp 3: Grp 1 Indiv: Grp Mean Using FR_{sum} (unitless)	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.	RBA Grp 3: Grp 1 Indiv: Grp Mean Using FR_{sum} (unitless)
2,3,7,8-TCDD	0.0039	0.0014	0.028	0.013	0.032	0.013	18%	0.0042	0.0008	0.034	0.006	0.038	0.006	22%
1,2,3,7,8-PeCDD	0.0043	0.0023	0.040	0.018	0.044	0.018	24%	0.0069	0.0014	0.057	0.010	0.064	0.011	34%
1,2,3,6,7,8-HxCDD	0.0070	0.0037	0.073	0.042	0.080	0.043	38%	0.0113	0.0027	0.084	0.029	0.095	0.029	45%
1,2,3,4,6,7,8-HpCDD	0.0185	0.0063	0.046	0.011	0.064	0.016	55%	0.0185	0.0063	0.046	0.011	0.064	0.016	55%
2,3,4,7,8-PeCDF	0.0440	0.0121	0.042	0.024	0.086	0.025	32%	0.0440	0.0121	0.067	0.014	0.111	0.018	41%
2,3,7,8-TCDD	0.0036		0.012		0.016		0.0898	0.0036		0.024		0.028		0.1580
1,2,3,7,8-PeCDD	0.0035		0.021		0.024		0.1308	0.0070		0.041		0.048		0.2573
1,2,3,6,7,8-HxCDD	0.0065		0.021		0.028		0.1338	0.0130		0.043		0.056		0.2649
1,2,3,4,6,7,8-HpCDD	0.0106		0.030		0.041		0.3457	0.0106		0.030		0.041		0.3457
2,3,4,7,8-PeCDF	0.0281		0.034		0.062		0.2293	0.0281		0.068		0.096		0.3544
2,3,7,8-TCDD	0.0041		0.016		0.020		0.1142	0.0041		0.032		0.036		0.2042
1,2,3,7,8-PeCDD	0.0083		0.030		0.038		0.2069	0.0083		0.060		0.068		0.3631
1,2,3,6,7,8-HxCDD	0.0132		0.085		0.099		0.4753	0.0132		0.085		0.099		0.4704
1,2,3,4,6,7,8-HpCDD	0.0245		0.043		0.068		0.5751	0.0245		0.043		0.068		0.5751
2,3,4,7,8-PeCDF	0.0566		0.028		0.085		0.3133	0.0566		0.056		0.113		0.4171
2,3,7,8-TCDD	0.0017		0.036		0.038		0.2177	0.0033		0.036		0.040		0.2260
1,2,3,7,8-PeCDD	0.0023		0.051		0.053		0.2887	0.0045		0.051		0.056		0.2961
1,2,3,6,7,8-HxCDD	0.0039		0.035		0.039		0.1878	0.0077		0.070		0.078		0.3717
1,2,3,4,6,7,8-HpCDD	0.0147		0.043		0.058		0.4928	0.0147		0.043		0.058		0.4928
2,3,4,7,8-PeCDF	0.0390		0.026		0.065		0.2408	0.0390		0.052		0.091		0.3372
2,3,7,8-TCDD	0.0053		0.037		0.042		0.2401	0.0053		0.037		0.042		0.2388
1,2,3,7,8-PeCDD	0.0034		0.067		0.070		0.3778	0.0069		0.067		0.073		0.3899
1,2,3,6,7,8-HxCDD	0.0044		0.114		0.118		0.5685	0.0089		0.114		0.123		0.5839
1,2,3,4,6,7,8-HpCDD	0.0176		0.059		0.076		0.6481	0.0176		0.059		0.076		0.6481
2,3,4,7,8-PeCDF	0.0404		0.084		0.124		0.4603	0.0404		0.084		0.124		0.4603
2,3,7,8-TCDD	0.0046		0.040		0.044		0.2529	0.0046		0.040		0.044		0.2516
1,2,3,7,8-PeCDD	0.0038		0.032		0.036		0.1958	0.0077		0.065		0.072		0.3852
1,2,3,6,7,8-HxCDD	0.0067		0.109		0.116		0.5567	0.0135		0.109		0.122		0.5831
1,2,3,4,6,7,8-HpCDD	0.0251		0.054		0.079		0.6703	0.0251		0.054		0.079		0.6703
2,3,4,7,8-PeCDF	0.0557		0.039		0.094		0.3493	0.0557		0.077		0.133		0.4925

Table D-11. (cont.)

	Midland Reference Oil (Group 1)														
					Using Mean BW										
Analyte	Mean Conc.[b] (ng/mL)	Total Volume Oil Mixture (mL)	Pig ID	Total Dose (ng)	Average Daily Dose (ng/kg BW/d)	Average Daily Dose S.D.	Mean Body Weight (kg)	Terminal Body Weight (kg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)	Fat Weight Using term. BW (g)	Fat Conc. (pg/g)	WHO TEF (unitless)	Using 1/2 DL Liver TEQ (pg/g)	Using DL Liver TEQ (pg/g)
2,3,7,8-TCDD	0.389	120	Grp 1 Mean	46.7	0.0807	0.0038	19.31	28.57	633.3		1,926		1		
1,2,3,7,8-PeCDD	0.177	120	Grp 1 Mean	21.2	0.0367	0.0017	19.31	28.57	633.3		1,926		1		
1,2,3,6,7,8-HxCDD	0.232	120	Grp 1 Mean	27.8	0.0482	0.0023	19.31	28.57	633.3		1,926		0.1		
1,2,3,4,6,7,8-HpCDD	2.98	120	Grp 1 Mean	358	0.619	0.029	19.31	28.57	633.3		1,926		0.01		
2,3,4,7,8-PeCDF	0.098	120	Grp 1 Mean	11.8	0.0203	0.0010	19.31	28.57	633.3		1,926		0.5		
2,3,7,8-TCDD	0.389	120	415	46.7	0.082		18.96	28.55	594.8	0.711 *Um*	1,924	3.53	1	0.356	0.711
1,2,3,7,8-PeCDD	0.177	120	415	21.2	0.037		18.96	28.55	594.8	0.553 *J*	1,924	1.71 *J*	1	0.553	0.553
1,2,3,6,7,8-HxCDD	0.232	120	415	27.8	0.049		18.96	28.55	594.8	0.993 *Um*	1,924	2.81 *J*	0.1	0.050	0.099
1,2,3,4,6,7,8-HpCDD	2.98	120	415	358	0.629		18.96	28.55	594.8	15.3	1,924	13.7	0.01	0.153	0.153
2,3,4,7,8-PeCDF	0.098	120	415	11.8	0.021		18.96	28.55	594.8	1.77 *J*	1,924	1.07 *J*	0.5	0.885	0.885
2,3,7,8-TCDD	0.389	120	419	46.7	0.077		20.30	30.40	754.6	0.839	2,049	4.04	1	0.839	0.839
1,2,3,7,8-PeCDD	0.177	120	419	21.2	0.035		20.30	30.40	754.6	0.427 *J*	2,049	1.67 *J*	1	0.427	0.427
1,2,3,6,7,8-HxCDD	0.232	120	419	27.8	0.046		20.30	30.40	754.6	0.629 *J*	2,049	2.36 *J*	0.1	0.063	0.063
1,2,3,4,6,7,8-HpCDD	2.98	120	419	358	0.587		20.30	30.40	754.6	9.69	2,049	15.5	0.01	0.097	0.097
2,3,4,7,8-PeCDF	0.098	120	419	11.8	0.019		20.30	30.40	754.6	1.24 *J*	2,049	0.979 *J*	0.5	0.620	0.620
2,3,7,8-TCDD	0.389	120	435	46.7	0.086		18.16	26.35	500.8	1.03	1,776	4.10	1	1.030	1.030
1,2,3,7,8-PeCDD	0.177	120	435	21.2	0.039		18.16	26.35	500.8	0.662 *J*	1,776	2.11 *J*	1	0.662	0.662
1,2,3,6,7,8-HxCDD	0.232	120	435	27.8	0.051		18.16	26.35	500.8	1.25 *J*	1,776	2.74 *J*	0.1	0.125	0.125
1,2,3,4,6,7,8-HpCDD	2.98	120	435	358	0.656		18.16	26.35	500.8	26.7	1,776	20.3	0.01	0.267	0.267
2,3,4,7,8-PeCDF	0.098	120	435	11.8	0.022		18.16	26.35	500.8	2.08 *J*	1,776	1.04 *J*	0.5	1.040	1.040
2,3,7,8-TCDD	0.389	120	439	46.7	0.082		18.95	28.30	660.8	0.797	1,907	4.54	1	0.797	0.797
1,2,3,7,8-PeCDD	0.177	120	439	21.2	0.037		18.95	28.30	660.8	0.475 *Um*	1,907	2.30 *J*	1	0.238	0.475
1,2,3,6,7,8-HxCDD	0.232	120	439	27.8	0.049		18.95	28.30	660.8	1.05 *J*	1,907	3.24 *J*	0.1	0.105	0.105
1,2,3,4,6,7,8-HpCDD	2.98	120	439	358	0.629		18.95	28.30	660.8	20.4	1,907	20.8	0.01	0.204	0.204
2,3,4,7,8-PeCDF	0.098	120	439	11.8	0.021		18.95	28.30	660.8	2.07 *J*	1,907	1.24 *J*	0.5	1.035	1.035
2,3,7,8-TCDD	0.389	120	443	46.7	0.077		20.15	29.25	655.7	0.754	1,971	3.82	1	0.754	0.754
1,2,3,7,8-PeCDD	0.177	120	443	21.2	0.035		20.15	29.25	655.7	0.508 *Um*	1,971	1.78 *J*	1	0.254	0.508
1,2,3,6,7,8-HxCDD	0.232	120	443	27.8	0.046		20.15	29.25	655.7	0.924 *J*	1,971	2.50 *J*	0.1	0.092	0.092
1,2,3,4,6,7,8-HpCDD	2.98	120	443	358	0.591		20.15	29.25	655.7	13.2	1,971	12.6	0.01	0.132	0.132
2,3,4,7,8-PeCDF	0.098	120	443	11.8	0.019		20.15	29.25	655.7	1.87 *J*	1,971	1.01 *J*	0.5	0.935	0.935

Table D-11. (cont.)

	Midland Reference Oil (Group 1)											
	Using 1/2 DL						Using DL					
Analyte	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.
2,3,7,8-TCDD	0.010	0.003	0.165	0.016	0.175	0.019	0.011	0.002	0.165	0.016	0.176	0.017
1,2,3,7,8-PeCDD	0.012	0.004	0.173	0.020	0.185	0.018	0.015	0.000	0.173	0.020	0.188	0.020
1,2,3,6,7,8-HxCDD	0.019	0.006	0.188	0.021	0.208	0.022	0.021	0.003	0.188	0.021	0.210	0.023
1,2,3,4,6,7,8-HpCDD	0.029	0.008	0.089	0.018	0.118	0.024	0.029	0.008	0.089	0.018	0.118	0.024
2,3,4,7,8-PeCDF	0.096	0.015	0.175	0.016	0.270	0.029	0.096	0.015	0.175	0.016	0.270	0.029
2,3,7,8-TCDD	0.005		0.146		0.150		0.009		0.146		0.155	
1,2,3,7,8-PeCDD	0.015		0.155		0.170		0.015		0.155		0.170	
1,2,3,6,7,8-HxCDD	0.011		0.194		0.205		0.021		0.194		0.215	
1,2,3,4,6,7,8-HpCDD	0.025		0.074		0.099		0.025		0.074		0.099	
2,3,4,7,8-PeCDF	0.090		0.175		0.265		0.090		0.175		0.265	
2,3,7,8-TCDD	0.014		0.177		0.191		0.014		0.177		0.191	
1,2,3,7,8-PeCDD	0.015		0.161		0.176		0.015		0.161		0.176	
1,2,3,6,7,8-HxCDD	0.017		0.174		0.191		0.017		0.174		0.191	
1,2,3,4,6,7,8-HpCDD	0.020		0.089		0.109		0.020		0.089		0.109	
2,3,4,7,8-PeCDF	0.080		0.171		0.250		0.080		0.171		0.250	
2,3,7,8-TCDD	0.011		0.156		0.167		0.011		0.156		0.167	
1,2,3,7,8-PeCDD	0.016		0.176		0.192		0.016		0.176		0.192	
1,2,3,6,7,8-HxCDD	0.022		0.175		0.197		0.022		0.175		0.197	
1,2,3,4,6,7,8-HpCDD	0.037		0.101		0.138		0.037		0.101		0.138	
2,3,4,7,8-PeCDF	0.089		0.157		0.246		0.089		0.157		0.246	
2,3,7,8-TCDD	0.011		0.186		0.197		0.011		0.186		0.197	
1,2,3,7,8-PeCDD	0.007		0.207		0.214		0.015		0.207		0.221	
1,2,3,6,7,8-HxCDD	0.025		0.222		0.247		0.025		0.222		0.247	
1,2,3,4,6,7,8-HpCDD	0.038		0.111		0.149		0.038		0.111		0.149	
2,3,4,7,8-PeCDF	0.116		0.201		0.317		0.116		0.201		0.317	
2,3,7,8-TCDD	0.011		0.161		0.172		0.011		0.161		0.172	
1,2,3,7,8-PeCDD	0.008		0.165		0.173		0.016		0.165		0.181	
1,2,3,6,7,8-HxCDD	0.022		0.177		0.199		0.022		0.177		0.199	
1,2,3,4,6,7,8-HpCDD	0.024		0.069		0.094		0.024		0.069		0.094	
2,3,4,7,8-PeCDF	0.104		0.169		0.274		0.104		0.169		0.274	

Note: One-half of the detection limit was used in calculations for non-detect concentrations.

[a] Average of triplicate samples.

[b] Average of duplicate analyses.

U – nondetect; value represents detection limit

Um – nondetect; value represents estimated maximum possible concentration (EMPC)

Table D-12. Tissue concentrations, doses, and RBA calculations for the swine pilot study: Tittabawassee River flood plain soil

	Tittabawassee River Flood Plain Soil (Group 4)																
	Imerman Park 2 (THT02769)					Using Mean BW											
Analyte	Soil Mean Conc.[a] (pg/g)	% of TEQ	Soil Dose Daily Mass of Chemical (ng/day)	Pig ID	Total Dose (ng)	Average Daily Dose (ng/kg BW/d)	Average Daily Dose S.D.	Mean Body Weight (kg)	Terminal Body Weight (kg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)	Fat Weight Using term BW (g)	Fat Conc. (pg/g)	WHO TEF (unitless)	Using 1/2 DL Liver TEQ (pg/g)	Using DL Liver TEQ (pg/g)
2,3,7,8-TCDF	2,150	25%	21.5	Grp 4 Mean	645	1.12	0.045	19.20 [b]	28.30 [b]	666.2 [b]		1,907		0.1		
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	Grp 4 Mean	323	0.561	0.023	19.20 [b]	28.30 [b]	666.2 [b]		1,907		0.05		
2,3,4,7,8-PeCDF	883	52%	8.83	Grp 4 Mean	265	0.460	0.018	19.20 [b]	28.30 [b]	666.2 [b]		1,907		0.5		
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	Grp 4 Mean	216	0.375	0.015	19.20 [b]	28.30 [b]	666.2 [b]		1,907		0.1		
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	Grp 4 Mean	49.1	0.0853	0.0034	19.20 [b]	28.30 [b]	666.2 [b]		1,907		0.1		
2,3,7,8-TCDF	2,150	25%	21.5	427	645	1.054		20.40	29.70	566.9	0.175 *U*	2,002	0.949	0.1	0.0088	0.0175
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	427	323	0.528		20.40	29.70	566.9	0.233 *U*	2,002	0.54 *J*	0.05	0.0058	0.0117
2,3,4,7,8-PeCDF	883	52%	8.83	427	265	0.433		20.40	29.70	566.9	12.3	2,002	4.91	0.5	6.1500	6.1500
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	427	216	0.353		20.40	29.70	566.9	8.38	2,002	6.49	0.1	0.8380	0.8380
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	427	49.1	0.080		20.40	29.70	566.9	2.79	2,002	1.46 *J*	0.1	0.2790	0.2790
2,3,7,8-TCDF	2,150	25%	21.5	428	640 [c]	1.151		18.67	27.60	656.1	0.221 *U*	1,860	0.983	0.1	0.0111	0.0221
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	428	320 [c]	0.576		18.67	27.60	656.1	0.259 *U*	1,860	0.834 *J*	0.05	0.0065	0.0130
2,3,4,7,8-PeCDF	883	52%	8.83	428	263 [c]	0.473		18.67	27.60	656.1	10.6	1,860	6.9	0.5	5.3000	5.3000
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	428	214 [c]	0.385		18.67	27.60	656.1	6.89	1,860	8.46	0.1	0.6890	0.6890
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	428	48.7 [c]	0.088		18.67	27.60	656.1	2.36 *J*	1,860	1.79 *J*	0.1	0.2360	0.2360
2,3,7,8-TCDF	2,150	25%	21.5	440	645	1.144		18.80	28.00	795.7	0.229 *U*	1,887	0.976	0.1	0.0115	0.0229
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	440	323	0.572		18.80	28.00	795.7	0.21 *U*	1,887	0.652 *J*	0.05	0.0053	0.0105
2,3,4,7,8-PeCDF	883	52%	8.83	440	265	0.470		18.80	28.00	795.7	9.15	1,887	5.94	0.5	4.5750	4.5750
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	440	216	0.383		18.80	28.00	795.7	6.42	1,887	7.79	0.1	0.6420	0.6420
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	440	49.1	0.087		18.80	28.00	795.7	2.06 *J*	1,887	1.69 *J*	0.1	0.2060	0.2060
2,3,7,8-TCDF	2,150	25%	21.5	441	645	1.135		18.95	27.90	646	0.27 *U*	1,880	0.665 *J*	0.1	0.0135	0.0270
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	441	323	0.568		18.95	27.90	646	0.242 *U*	1,880	0.439 *Um*	0.05	0.0061	0.0121
2,3,4,7,8-PeCDF	883	52%	8.83	441	265	0.466		18.95	27.90	646	11.8	1,880	5.54	0.5	5.9000	5.9000
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	441	216	0.380		18.95	27.90	646	8.85	1,880	7.81	0.1	0.8850	0.8850
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	441	49.1	0.086		18.95	27.90	646	2.73	1,880	1.71 *J*	0.1	0.2730	0.2730
2,3,7,8-TCDF	2,150	25%	21.5	444	<538 [d]			16.50	22.00	533.2	0.178 *U*	1,483	0.318 *U*	0.1	0.0089	0.0178
1,2,3,7,8-PeCDF	1,076	6.3%	10.8	444	<269 [d]			16.50	22.00	533.2	0.312 *U*	1,483	0.304 *U*	0.05	0.0078	0.0156
2,3,4,7,8-PeCDF	883	52%	8.83	444	<221 [d]			16.50	22.00	533.2	3.71	1,483	1.99 *Um*	0.5	1.8550	1.8550
1,2,3,4,7,8-HxCDF	719	8.5%	7.19	444	<180 [d]			16.50	22.00	533.2	1.78 *J*	1,483	2.54 *J*	0.1	0.1780	0.1780
1,2,3,6,7,8-HxCDF	164 *D*	1.9%	1.64	444	<40.9 [d]			16.50	22.00	533.2	0.574 *J*	1,483	0.599 *Um*	0.1	0.0574	0.0574

Table D-12. (cont.)

	Tittabawassee River Flood Plain Soil (Group 4)													
	Using 1/2 DL							Using DL						
Analyte	Fraction Retained in Liver[b] FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat[b] FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat[b] FR_{sum} (unitless)	FR_{sum} S.D.	RBA Grp 4 : Grp 2 Indiv: Grp Mean Using FR_{sum} (unitless)	Fraction Retained in Liver[b] FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat[b] FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat[b] FR_{sum} (unitless)	FR_{sum} S.D.	RBA Grp 4 : Grp 2 Indiv: Grp Mean Using FR_{sum} (unitless)
2,3,7,8-TCDF	0.0001	0.00003	0.0026	0.0005	0.0028	0.0005	22%	0.0002	0.00006	0.0026	0.0005	0.0029	0.0004	23%
1,2,3,7,8-PeCDF	0.0002	0.00003	0.0033	0.0015	0.0036	0.0015	30%	0.0005	0.00005	0.0036	0.0010	0.0041	0.0010	34%
2,3,4,7,8-PeCDF	0.0273	0.0011	0.0419	0.0051	0.0692	0.0049	27%	0.0273	0.0011	0.0419	0.0051	0.0692	0.0049	27%
1,2,3,4,7,8-HxCDF	0.0233	0.0024	0.0675	0.0055	0.0908	0.0059	35%	0.0233	0.0024	0.0675	0.0055	0.0908	0.0059	35%
1,2,3,6,7,8-HxCDF	0.0333	0.0019	0.0646	0.0037	0.0979	0.0043	37%	0.0333	0.0019	0.0646	0.0037	0.0979	0.0043	37%
2,3,7,8-TCDF	0.0001		0.0029		0.0030		0.2426	0.0002		0.0029		0.0031		0.2487
1,2,3,7,8-PeCDF	0.0002		0.0033		0.0036		0.2974	0.0004		0.0033		0.0038		0.3095
2,3,4,7,8-PeCDF	0.0263		0.0371		0.0635		0.2500	0.0263		0.0371		0.0635		0.2500
1,2,3,4,7,8-HxCDF	0.0220		0.0602		0.0822		0.3208	0.0220		0.0602		0.0822		0.3208
1,2,3,6,7,8-HxCDF	0.0322		0.0595		0.0917		0.3503	0.0322		0.0595		0.0917		0.3503
2,3,7,8-TCDF	0.0001		0.0029		0.0030		0.2383	0.0002		0.0029		0.0031		0.2474
1,2,3,7,8-PeCDF	0.0003		0.0048		0.0051		0.4275	0.0005		0.0048		0.0054		0.4425
2,3,4,7,8-PeCDF	0.0265		0.0488		0.0753		0.2967	0.0265		0.0488		0.0753		0.2967
1,2,3,4,7,8-HxCDF	0.0211		0.0735		0.0946		0.3691	0.0211		0.0735		0.0946		0.3691
1,2,3,6,7,8-HxCDF	0.0318		0.0683		0.1001		0.3822	0.0318		0.0683		0.1001		0.3822
2,3,7,8-TCDF	0.0001		0.0029		0.0030		0.2405	0.0003		0.0029		0.0031		0.2519
1,2,3,7,8-PeCDF	0.0003		0.0038		0.0041		0.3407	0.0005		0.0038		0.0043		0.3566
2,3,4,7,8-PeCDF	0.0275		0.0423		0.0698		0.2752	0.0275		0.0423		0.0698		0.2752
1,2,3,4,7,8-HxCDF	0.0237		0.0681		0.0918		0.3582	0.0237		0.0681		0.0918		0.3582
1,2,3,6,7,8-HxCDF	0.0334		0.0650		0.0983		0.3755	0.0334		0.0650		0.0983		0.3755
2,3,7,8-TCDF	0.0001		0.0019		0.0021		0.1665	0.0003		0.0019		0.0022		0.1773
1,2,3,7,8-PeCDF	0.0002		0.0013		0.0015		0.1273	0.0005		0.0026		0.0030		0.2505
2,3,4,7,8-PeCDF	0.0288		0.0393		0.0681		0.2685	0.0288		0.0393		0.0681		0.2685
1,2,3,4,7,8-HxCDF	0.0265		0.0681		0.0945		0.3690	0.0265		0.0681		0.0945		0.3690
1,2,3,6,7,8-HxCDF	0.0359		0.0655		0.1014		0.3872	0.0359		0.0655		0.1014		0.3872
2,3,7,8-TCDF	0.0001		0.0004		0.0005			0.0002		0.0009		0.0011		
1,2,3,7,8-PeCDF	0.0003		0.0008		0.0011			0.0006		0.0017		0.0023		
2,3,4,7,8-PeCDF	0.0090		0.0067		0.0157			0.0090		0.0134		0.0223		
1,2,3,4,7,8-HxCDF	0.0053		0.0209		0.0262			0.0053		0.0209		0.0262		
1,2,3,6,7,8-HxCDF	0.0075		0.0109		0.0183			0.0075		0.0217		0.0292		

Table D-12. (cont.)

	Tittabawassee River Flood Plain Reference Oil (Group 2)														
					Using Mean BW										
Analyte	Mean Conc.[e] (ng/mL)	Total Volume Oil Mixture (mL)	Pig ID	Total Dose (ng)	Average Daily Dose (ng/kg BW/d)	Average Daily Dose S.D.	Mean Body Weight (kg)	Terminal Body Weight (kg)	Liver Weight (mean) (g)	Liver Conc. (pg/g)	Fat Weight Using term BW (g)	Fat Conc. (pg/g)	WHO TEF (unitless)	Using 1/2 DL Liver TEQ (pg/g)	Using DL Liver TEQ (pg/g)
2,3,7,8-TCDF	4.90	120	Grp 2 Mean	588	1.08	0.036	18.18	27.17	584.7		1,831		0.1		
1,2,3,7,8-PeCDF	2.94	120	Grp 2 Mean	353	0.647	0.021	18.18	27.17	584.7		1,831		0.05		
2,3,4,7,8-PeCDF	2.50	120	Grp 2 Mean	300	0.550	0.018	18.18	27.17	584.7		1,831		0.5		
1,2,3,4,7,8-HxCDF	1.99	120	Grp 2 Mean	239	0.438	0.014	18.18	27.17	584.7		1,831		0.1		
1,2,3,6,7,8-HxCDF	0.490	120	Grp 2 Mean	58.8	0.108	0.0036	18.18	27.17	584.7		1,831		0.1		
2,3,7,8-TCDF	4.90	120	403	588	1.100		17.81	26.90	621.4	0.635	1,813	4.36	0.1	0.0635	0.0635
1,2,3,7,8-PeCDF	2.94	120	403	353	0.660		17.81	26.90	621.4	0.360 *Um*	1,813	2.48 *J*	0.05	0.009	0.018
2,3,4,7,8-PeCDF	2.50	120	403	300	0.561		17.81	26.90	621.4	55.6	1,813	27.4	0.5	27.8	27.8
1,2,3,4,7,8-HxCDF	1.99	120	403	239	0.447		17.81	26.90	621.4	28.1	1,813	26.6	0.1	2.81	2.81
1,2,3,6,7,8-HxCDF	0.490	120	403	58.8	0.110		17.81	26.90	621.4	9.35	1,813	5.89	0.1	0.935	0.935
2,3,7,8-TCDF	4.90	120	410	588	1.099		17.84	26.15	568.5	0.712	1,763	2.78	0.1	0.0712	0.0712
1,2,3,7,8-PeCDF	2.94	120	410	353	0.659		17.84	26.15	568.5	0.286 *Um*	1,763	1.74 *J*	0.05	0.00715	0.0143
2,3,4,7,8-PeCDF	2.50	120	410	300	0.561		17.84	26.15	568.5	70.0	1,763	20.1	0.5	35.0	35.0
1,2,3,4,7,8-HxCDF	1.99	120	410	239	0.446		17.84	26.15	568.5	37.1	1,763	21.9	0.1	3.71	3.71
1,2,3,6,7,8-HxCDF	0.490	120	410	58.8	0.110		17.84	26.15	568.5	12.9	1,763	4.57 *J*	0.1	1.29	1.29
2,3,7,8-TCDF	4.90	120	425	588	1.038		18.88	27.80	560.1	0.549	1,874	4.19	0.1	0.0549	0.0549
1,2,3,7,8-PeCDF	2.94	120	425	353	0.623		18.88	27.80	560.1	0.275 *J*	1,874	2.65 *J*	0.05	0.01375	0.01375
2,3,4,7,8-PeCDF	2.50	120	425	300	0.530		18.88	27.80	560.1	51.8	1,874	29.9	0.5	25.9	25.9
1,2,3,4,7,8-HxCDF	1.99	120	425	239	0.422		18.88	27.80	560.1	27.2	1,874	28	0.1	2.72	2.72
1,2,3,6,7,8-HxCDF	0.490	120	425	58.8	0.104		18.88	27.80	560.1	8.75	1,874	5.93	0.1	0.875	0.875
2,3,7,8-TCDF	4.90	120	432	588	1.043		18.80	28.40	572.7	0.577	1,914	4.28	0.1	0.0577	0.0577
1,2,3,7,8-PeCDF	2.94	120	432	353	0.626		18.80	28.40	572.7	0.241 *Um*	1,914	2.19 *J*	0.05	0.006025	0.01205
2,3,4,7,8-PeCDF	2.50	120	432	300	0.532		18.80	28.40	572.7	48.9	1,914	22.6	0.5	24.45	24.45
1,2,3,4,7,8-HxCDF	1.99	120	432	239	0.424		18.80	28.40	572.7	27.6	1,914	23.4	0.1	2.76	2.76
1,2,3,6,7,8-HxCDF	0.490	120	432	58.8	0.104		18.80	28.40	572.7	9.92	1,914	5.26	0.1	0.992	0.992
2,3,7,8-TCDF	4.90	120	447	588	1.114		17.59	26.60	601.0	0.298 *J*	1,793	3.44	0.1	0.0298	0.0298
1,2,3,7,8-PeCDF	2.94	120	447	353	0.669		17.59	26.60	601.0	0.274 *U*	1,793	2.15 *J*	0.05	0.00685	0.0137
2,3,4,7,8-PeCDF	2.50	120	447	300	0.569		17.59	26.60	601.0	40.6	1,793	22.6	0.5	20.3	20.3
1,2,3,4,7,8-HxCDF	1.99	120	447	239	0.453		17.59	26.60	601.0	20.5	1,793	22.3	0.1	2.05	2.05
1,2,3,6,7,8-HxCDF	0.490	120	447	58.8	0.111		17.59	26.60	601.0	7.04	1,793	5.09	0.1	0.704	0.704

Table D-12. (cont.)

	Tittabawassee River Flood Plain Reference Oil (Group 2)											
	Using 1/2 DL						Using DL					
Analyte	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.	Fraction Retained in Liver FR_{liver} (unitless)	FR_{liver} S.D.	Fraction Retained in Fat FR_{fat} (unitless)	FR_{fat} S.D.	Fraction Retained Liver+Fat FR_{sum} (unitless)	FR_{sum} S.D.
2,3,7,8-TCDF	0.0005	0.0002	0.012	0.002	0.012	0.002	0.0005	0.0002	0.012	0.002	0.012	0.002
1,2,3,7,8-PeCDF	0.0003	0.0001	0.012	0.002	0.012	0.002	0.0005	0.0001	0.012	0.002	0.012	0.002
2,3,4,7,8-PeCDF	0.104	0.020	0.150	0.027	0.254	0.029	0.104	0.020	0.150	0.027	0.254	0.029
1,2,3,4,7,8-HxCDF	0.069	0.013	0.188	0.024	0.256	0.025	0.069	0.013	0.188	0.024	0.256	0.025
1,2,3,6,7,8-HxCDF	0.095	0.020	0.167	0.021	0.262	0.021	0.095	0.020	0.167	0.021	0.262	0.021
2,3,7,8-TCDF	0.0007		0.013		0.014		0.0007		0.013		0.014	
1,2,3,7,8-PeCDF	0.0003		0.013		0.013		0.0006		0.013		0.013	
2,3,4,7,8-PeCDF	0.115		0.166		0.281		0.1152		0.166		0.281	
1,2,3,4,7,8-HxCDF	0.073		0.202		0.275		0.0731		0.202		0.275	
1,2,3,6,7,8-HxCDF	0.099		0.182		0.280		0.0988		0.182		0.280	
2,3,7,8-TCDF	0.0007		0.008		0.009		0.0007		0.008		0.009	
1,2,3,7,8-PeCDF	0.0002		0.009		0.009		0.0005		0.009		0.009	
2,3,4,7,8-PeCDF	0.133		0.118		0.251		0.1327		0.118		0.251	
1,2,3,4,7,8-HxCDF	0.088		0.162		0.250		0.0883		0.162		0.250	
1,2,3,6,7,8-HxCDF	0.125		0.137		0.262		0.1247		0.137		0.262	
2,3,7,8-TCDF	0.0005		0.013		0.014		0.0005		0.013		0.014	
1,2,3,7,8-PeCDF	0.0004		0.014		0.015		0.0004		0.014		0.015	
2,3,4,7,8-PeCDF	0.097		0.187		0.283		0.0967		0.187		0.283	
1,2,3,4,7,8-HxCDF	0.064		0.220		0.283		0.0638		0.220		0.283	
1,2,3,6,7,8-HxCDF	0.083		0.189		0.272		0.0833		0.189		0.272	
2,3,7,8-TCDF	0.0006		0.014		0.014		0.0006		0.014		0.014	
1,2,3,7,8-PeCDF	0.0002		0.012		0.012		0.0004		0.012		0.012	
2,3,4,7,8-PeCDF	0.093		0.144		0.238		0.0934		0.144		0.238	
1,2,3,4,7,8-HxCDF	0.066		0.188		0.254		0.0662		0.188		0.254	
1,2,3,6,7,8-HxCDF	0.097		0.171		0.268		0.0966		0.171		0.268	
2,3,7,8-TCDF	0.0003		0.010		0.011		0.0003		0.010		0.011	
1,2,3,7,8-PeCDF	0.0002		0.011		0.011		0.0005		0.011		0.011	
2,3,4,7,8-PeCDF	0.081		0.135		0.216		0.0813		0.135		0.216	
1,2,3,4,7,8-HxCDF	0.052		0.167		0.219		0.0516		0.167		0.219	
1,2,3,6,7,8-HxCDF	0.072		0.155		0.227		0.0720		0.155		0.227	

(notes on following page)

Table D-12. (cont.)

Note: Calculations were performed using one-half the detection limit for non-detects.
U – nondetect; value represents detection limit
Um – nondetect; value represents estimated maximum possible concentration (EMPC)

[a] Average of triplicate samples.
[b] Excluding results from swine #444, who became sick and was found dead on Study Day 25
[c] Total dosed material received by Pig 428 was adjusted downward slightly per notes in log book.
[d] Swine 444 was offered a maximum of 25 doses (from Study Day 0–24). He did not eat all of the doses he was given because of illness. However, additional details of the total dosed material were not estimated because results associated with this animal were excluded from final calculations.
[e] Average of duplicate analyses.